NOVEL THERAPEUTIC INDICATION OF AZITHROMYCIN FOR TREATMENT OF NON-INFECTIVE INFLAMMATORY DISEASES

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Abstract

The invention relates to the use of 9-deoxo-9-dihydro-9a-methyl-9a-aza-9a-homoerythromycin. A (generic name: azithromycin) for the therapy of neutrophil-dominated non-infective inflammatory diseases, pharmaceutical compositions containing azithromycin for enteral or parenteral administration and methods for the production of these pharmaceutical compositons.

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NEW THERAPEUTIC APPLICATION OF AZITHROMYCIN IN THE TREATMENT OF NONINFECTIOUS INFLAMATORY DISEASES

DESCRIPTION OF INVENTION

The present invention relates to the usage of 9-deoxo-9-dihydro-9a-methyl-9a-aza-9a-homoerythromicin A (generic name: azithromycin) for treating noninfectious inflammatory diseases with neutrophil prevalence, pharmaceutical compositions containing azithromycin for enteral or parenteral application, and methods for preparing those pharmaceutical compositions.

Majority of inflammatory diseases are characterised by an abnormal accumulation of inflammatory cells including monocytes/macrophages, granulocytes, plasma cells, lymphocytes, and platelets (thrombocytes). Together with endothelial tissue cells and fibroblasts, these inflammatory cells release a complex series of lipids, growing factors, cytokines and destructive enzymes that cause the local tissue damage.

Certain form of an inflammatory response is neutrophile inflammation being characterised by infiltration of inflamed tissue with neutrophile polymorphonuclear leukocytes (PMN), which are the most important component of host defence system. The tissue infection with extracellular bacteria represents a prototype for this inflammatory response. Besides, various non-infectious diseases are being characterised by extravascular recruitment of neutrophils. This group of inflammatory diseases include chronic obstructive pulmonary disease, adult respiratory distress syndrome, some forms of alveolitis caused by immune complexes, cystic fibrosis, bronchitis, bronchiectasis, emphysema, glomerulonephritis, active phases of rheumatoid arthritis, gout arthritis, ulcerous colitis, specific dermatoses like psoriasis and vasculitis. It is considered that at these conditions the neutrophils play the key role in formation of tissue lesion, which can, if being persistent, lead to irreversible destruction of normal tissue architecture with consequent organ dysfunction. That is why the tissue lesion is mostly caused by an activation of neutrophils with subsequent proteases release and increased production of oxygen radicals.

Chronic obstructive pulmonary disease (COPD) is basely the condition which is described like progressive development of airflow restriction that is not completely reversible (ATC, 1995). The majority of patients suffering from COPD have got three pathological conditions: bronchitis, emphysema and mucus accumulation. This disease is characterised by slow progressive and irreversible decrease in force expiratory volume in the first second of breathing out (FEV_1), with relative preservation of force vital capacity (FVC) (*Barnes, N. Engl. J. Med. (2000), 343(4): 269-280*). In asthma and COPD there is significant, but diverse, transformation of respiratory ways. The larger part of airflow obstruction is due to two major components, alveolar destruction (emphysema) and obstruction of small airways (chronic obstructive bronchitis). In COPD, it is predominantly characterised by the strong hyperplasia of mucous cells.

Smoking, air pollution and the other environmental factors are the major causes of sicknesses. Causal mechanism is not defined momentary but oxidative-antioxidative disorders are strongly implicated within a sickness development. COPD is the chronic inflammatory process which is significantly distinguished from the process noticed in asthma, having various inflammatory cells, mediators, inflammatory effects and responses to the treatment (*Keatings et al., Am. J. Respir. Crit Care Med. (1996), 153: 530-534*). Principally, the characteristic of this illness is neutrophil infiltration of patient's lung.

It seems that higher levels of pro-inflammatory cytokines like TNF-α, and particularly chemokines like IL-8 and GRO- α play very important role in the pathogenesis of this illness. The thromboxane synthesis in platelets (thrombocytes) is also enlarged in the patients suffering from COPD (*Keatings et al., Am. J. Respir. Crit Care Med. (1996), 153: 530-534; Stockley and Hill, Thorax (2000), 55(7): 629-630*). The most of the tissue damage is caused by an activation of neutrophils with subsequent release of (metallo)proteinase, and increased production of oxygen radicals (*Repine et al.., Am. J. Respir. Crit. Care Med. (1997), 156: 341-357; Barnes, Chest (2000), 117(2 Suppl): 10S-14S*).

The most of the therapeutic efforts were directed to symptom checking (*Barnes, Trends Pharm. Sci. (1998), 19(10): 415-423; Barnes, Am. J. Respir. Crit. Care Med. (1999) 160: S72-S-79; Hansel et al., Expert Opin. Investig. Drugs (2000) 9(1): 3-23*). The symptoms are usually equalised with airflow restriction and the bronchodilators represent treatment of choice. The prevention and the treating of complications, the prevention

of exacerbation and the life quality improvement as well as the life prolongation are also the principal goals cited in three international guides for COPD treatment (*Culpitt and Rogers, Exp. Ipin. Pharmacother,. (2000) 1(5): 1007-1020; Hay, Curr. Opin. Chem. Biol. (2000), 4: 412-419*). Basically, the most of the present therapeutical researches are directed to the mediators included in the regulation and the activation of neutrophils, or the lessening of the consequences of their undesired activation (*Stockey et al. Chest (2000), 117 (2 Suppl): 58S-62S*).

There is the great number of reports dealing with immuno-modulating action of macrolide antibiotics *in vitro (Labro, J. Antimicrob. Chemother. (1998), 41 (Suppl B): 37-46; Labro, Clin. Microb. Rev. (2000), 13(4): 615-650; Wales and Woodhead, Thorax (1999), 54 (Suppl. 2). S58-S62*). Macrolide antibiotics are macrocyclic compounds having for instance lactone ring with 12, 14, 16 or 17 members, and 1 to 3 carbohydrate units being linked to each others or to aglycon by glycoside bonds. The well-known macrolide antibiotics are, for example, carbomycin, erythromycin, leucomycin and spiramycin.

The most important findings relating to interaction of macrolides with phagocyte inflammatory cells in vitro refer to the inhibition of oxidant production by stimulated cells (*Labro et al., J. Antimicrob. Cemother. (1989), 24 (4): 561-572; Wmeki, Chest (1993), 104: 1191-1193; Vvenisch et al., Antimicrob. Agents Chemother. (1996), 40(9): 2039-2042*) and modulation of pro-inflammatory and antiinflammatory cytokines release from those cells (*Labro et al., J. Antimicrob. Chemother. (1989), 24 (4): 561-572; Khan et al., Internat. J. Antimicrob. Agents (1999), 11: 121-132; Morikawa et al., Antimicrob. Agents and Chermother (1996), 40(6): 1366-1370; Sugiyama et al., Eur. Respir. J. (1999), 14:113-1116*). Besides, several macrolides directly induce exocytosis (degranulation) of human neutrophils *in vitro (Abdelghaffae et al., Antimicrob. Agents Chemother. (1994), 38(7): 1548-1554; Vazifeh et al., Antimicrob. Agents Chemother. (1998), 42(8): 1944-1951)*. In an experimental model of pleuritis in rats caused by carrageenin, it was found that some of macrolide antibiotics like roxythromycin, clarithromycin and erythromycin, but not azithromycin, showed antiinflammatory activity which was probably dependent upon their ability of inhibition of pro-inflammatory mediators and cytokines production. In thiss acute inflammation model, the production of NO, TNF-α and PGE2 levels were significantly reduced by antibiotics pre-treatment (Lanario et al., J. Pharmacol. Exp. Ther. (2000), 292: 156-163).

The administration of erythromycin also produced an antiinflammatory activity in peritonitis in rats caused by zymosan (*Agen et al, Agents Action (1993), 38(1-2): 85-90*). It was reported that roxythromycin was effective in a reduction of acute inflammatory reaction by the mechanism, which was different from the conventional antiinflammatory medicaments as indomethacin. In another study, it was proven that roxythromycin was effective in the standard animal model being used for an evaluation of antiinflammatory drugs activity to edema of paw caused by carrageenin while clarithromycin and azithromycin showed a modest activity (*Scaglione and Rossini, J- Antimicrob. Chemother. (1998), 41, Suppl. B: 47-50*).

Some macrolide antibiotics, like erythromycin, clarithromycin and roxythromycin were already used like antiinflammatory drugs, especially for the treatment of diffusing panbronchitis. There are on disposition the reports about macrolide usage for the treatment of diseases like rheumatoid arthritis and cystic fibrosis (Arayssi et al., Programme and Abstracts of the 4th International conference on macrolides, azalides, streptogramins and ketolides, 21-23 January, Barcelona, Spain, Abstract 6, Singh, J. Assoc. Phys. India (1989), 37: 547; Jaffe et al., Lancet (1998), 351: 420). Regarding the relevant pharmacological effects of macrolides, it was reported that erythromycin inhibited a hypersecretion as a consequence of mucus and water secretion inhibition from epithelium cells. It also inhibits the accumulation of neutrophils in inflamed area because of inhibition of their binding to capillaries, the secretion of IL-8 from epithelium cells and the secretion of IL-8 and LTB_4 from the neutrophil itself. It advantageous activity in treatment of diffusing panbronchitis also includes reduction of superoxides production, and the reduction of proteolytic enzymes level in the lung.

It is proven that azithromycin significantly improves the lung function, but the mechanism explaining it is not clarified yet (*Jaffe wt al., Lancet (1998), 351: 42*), while it is reported for roxythromycin that it suppress the growth of nasal polyp fibroblasts (Nonaka et al., Am. J. Rhinol. (1999), 13: 267-272, Yamada et al., Am. J. Rhinol. (2000), 14: 143-148).

While there are firm proves in the published references that macrolides with 14 member ring, like erythromycin, clarithromycin and roxythromycin inhibit *in vitro* the production of IL-8 and the chemotaxis of neutrophils, even *in vitro* the proves that macrolides with 15 member ring as azithromycin have got the similar antiinflammatory activity are limited (Criqui et al., Eur. Respir. J. (2000), 15: 856-862).

In US patent 4,886,792 the inhibitory activity of macrolactone with 15 member ring on a degranulation of neutrophils was described, but they had no sugar substituents of azithromycin. It was reported that azithromycin induced apoptosis in human neutrophils *in vitro*, but it had no activity to oxidative metabolism or production of IL-8 (*Koch et al., J. Antimicrob. Chemother. (2000), 46: 19-26*). Only one study showed that azithromycin inhibited chemotaxis of neutrophils and active production of oxygen radicals *in vitro* (*Sugihara, Kansenshogaku Zasshi J. Jpn. Assoc. Infec. Dis. (1997), 71: 329-336*). It is also proven that azithromycin does not change the levels of TNF-α, IL-1β or IL-6 of alveolar macrophages or blood (Aubert et al., Pul. Pharmacol. Ther. (1998), 11: 263-269).

The possibility that azithromycin, because of its 15 member ring, did not posses required structure, that gave the antiinflammatory activity to macrolides with 14 member ring, was supposed and became more probable after it was noticed that macrolide with 16 member ring, like josamycin, did not reduced the production of IL-8 (*Takizawa et al., Am. J. Resp. Crit. Care Med. (1997), 156: 266-271; Criqui et al., Eur. Respir. J. (2000), 15: 856-862*).

In a comparison with macrolide antibiotics having 14 member ring, macrolide compounds with 15 member ring posses several advantages. For example, erythromycin, having the structure that is characterised by an aglicon 14 member ring, in acidic medium easily turns into anhydroerythromycin, which is inactive C-6/C-12 metabolite with spirochetal structure (Kurath et al., Experienta (1971), 27: 362). In distinction from its parent antibiotic erythromycin, azithromycin exhibits improved stability in acidic medium. Furthermore, azithromycin exhibits significantly higher concentration in tissues. Because of its improved *in vitro* activity to gram-negative micro organisms, even the possibility of single dosage per day was examined (Ratshema et al., Antimicrob. Agents Chemother. (1987), 31: 1939).

Because of it, the technical problem of this invention is insuring of improved methods, specially improved processes and applications useful for the therapy of non-infectious inflammatory diseases with neutrophil prevalence, wherein an active ingredient exhibits useful antiinflammatory activities of macrolide compounds having 14 member lactone ring, as well as the improved stability and the high concentration in the tissue of macrolide compounds having 15 member ring.

The present invention solves the above problem by using an active ingredient chosen from a group consisting of azithromycin, the pharmaceutically acceptable derivative thereof, the pharmaceutically acceptable hydrate thereof, the pharmaceutically acceptable complex or chelate thereof, and the pharmaceutically acceptable salt thereof, for the production of the pharmaceutical compositions for treating of non-infectious inflammatory diseases with neutrophil prevalence in humans and animals.

In distinction from limited azithromycin activity to neutrophil function *in vitro* described in the art, according to this invention, it was surprisingly found that azithromycin administered to the humans *in vivo* had large range of antiinflammatory activity and that it was exceptionally useful for the treatment of non-infectious inflammatory diseases characterised by an neutrophil infiltration and the tissue lesion in conjunction with neutrophils.

In the examination that was conducted on normal volunteers, the influence of azithromycin on selected parameters relevant to the inflammation was observed. It is found that the administration of azithromycin initiate degranulation of human neutrophils what is apparent from a considerable change of enzymatic concentration of principle azurophil granules, like myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase.

Biological relevance of MPO activity in granulocytes is the strong anti-microbe oxygen dependent activity in conjunction with a mobilisation of all granules in inflammatory granulocytes, especially after phagocyte stimulus by immune complexes. After administration of azithromycin, MPO activity in neutrophils in a blood smear was strongly decreased and turned back to the initial values after 28 days. Thus, it was found that the granulation that showed less MPO density in neutrophils, what was determined cytochemically, was in a relation with a lower concentration of MPO in lysate neutrophils measured by ELISA.

N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase are lyosomal enzymes, both placed in azurophil (principal and peroxidase-positive) granules of neutrophils. Since during the inflammation there is a degranulation of neutrophils, both enzymes are degranulation markers and can be used for the evaluation of neutrophil reactivity. Azithromycin studies showed that after the administration of azithromycin, the NAGA

activity in serum was significantly increased. Even 28 days after the final dose of azithromycin, the NAGA values in serum were still 70% higher than initial values. The increasing of NAGA in serum was followed by the decreasing of enzyme activity in PMN. The activity in PMN in serum showed no change in activity during the first day after the final doze of azithromycin, but it increased later on. 28 days after the final dose of azithromycin, the activity of β-glucuronidase was 40% higher than initial one. The activity of β-glucuronidase in PMN was reduced several hours after the final dose of azithromycin but it was increased thereafter. 28 days after the final dose of azithromycin, the activity of β-glucuronidase was much higher than initially.

In addition, according to the present invention, it is proven that azithromycin inhibits the production of reactive oxygen radicals in stimulated neutrophils, what is proven by the inhibition of chemoluminescence produced by stimulated neutrophils. The fact that azithromycin is the inhibitor of neutrophil oxidising bang it is further proven with the usage of cytochrome C test. The examinations also revealed that azithromycin had a long term activity on the concentration of the cell glutation peroxidase (GSHPx) and glutation reductase, two enzymes controlling the biological activity of free radicals being involved in a pathogenesis of a wide range of diseases. The production of free radicals and the disturbance in redox status can modulate the expression of wide range of inflammatory molecules, having influence on certain cell processes which lead to the inflammatory process. This is how azithromycin provides the bases for a treatment of various diseases like COPD wherein the production of free radicals becomes too excessive.

The studies also confirmed that azithromycin induced apoptosis, i.e. the programmed cell death of the certain cell types. The apoptosis represents important mechanism for completion of an immune response. The three day administration of azithromycin had delayed pro-apoptotic activity to the granulocytes, what was shown by morphology of the blood smear. The number of apoptotic cells reached maximum 28 days after the final dose of azithromycin, that indicated to the reduced number of active, potentially damaged neutrophils.

In the study, the other antiinflammatory activities of azithromycin were found. In distinction to prior researches (*Koch et al., J. Antimicrob. Chemother. (2000), 46: 19-26*) it was found, according to the present invention, that azithromycin had the explicit inhibitory activity to release of IL-8, as well as GRO-α. Interleuchin-8 (IL-8) is a member of CXC chemokines sub-family being specific for neutrophils. It is the strong chemotactic and activating factor for neutrophils (*Oppenheim, Ann. Rev. Immunol. (1999), 9:617*). The expression of IL-8 is a response to inflammatory excitation. IL-8 delays spontaneous and TNF-α mediated apoptosis of human neutrophils. In distinction to activity to IL-8, azithromycin gradually increased concentrations of cytokine IL-1 in serum, causing that the highest concentration of IL-1 was detected 24 hours after the final doze of azithromycin. However, the concentration of the other cytokine in serum, IL- 6, was being decreased constantly.

In distinction to prior reports (*Seman et al., J. Cardiovasc. Pharmacol. (2000), 36: 533-537*) wherein the treating with azithromycin did not significantly influence to the concentration of the soluble VCAM (sVCAM) in plasma, the studies conducted according to this invention clearly showed the significant level reduction of sVCAM in plasma already 24 hours after the treatment with azithromycin.

The results obtained according to this invention showed that three day treatment of normal human examinees, by the standard antibacterial regimen of azithromycin dosage, had an acute activity to neutrophil granular enzymes, oxidising bang, oxidising protective mechanisms and neutrophil chemokines and circulating IL-1, IL-6, IL-8, as well as delayed activity to apoptosis of neutrophils and soluble adhesive molecules.

According to this invention, thus, azithromycin can be used as the valuable prophylactic and/or therapeutic medicine for the treatment of non-infectious inflammatory diseases with neutrophil prevalence.

The following definition will be given in order to illustrate and define the meaning and the scope of various terms used for description of this invention.

The term "non-infectious inflammatory disease with neutrophil prevalence" refers to inflammatory diseases, disorders or conditions which take rise from a tissue damage, a chemical irradiation or immune processes, but not from an invasion of micro organisms like viruses, bacteria, moulds, protozoa, or similar, which are characterised by an infiltration of inflamed tissue with neutrophils being the first inflammatory cells entering into a tissue and amplifying an inflammatory response. In some non-infectious inflammatory diseases the neutrophils remain prevalent cell type within inflamed area, even then when the response is prolonged because of a permanent presence of infiltration and neutrophil activation stimulus. Thus, the examples are chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS), and neutrophil

dermatoses. The other non-infectious inflammatory diseases with neutrophil prevalence include the diseases having the stimulus for chronicity of pathology, which is not neutrophil dependant. For example, autoimmune diseases are mostly caused by development of immune responses to the normal structural body components and they include an activation of T lymphocytes, with possible production of the auto anti-body of B-lymphocytes. In rheumatoid arthritis (RA), for example, the immune reactions are directed against structural components of joints. However, in RA and other autoimmune diseases there are acute disease exacerbations, which are characterised by intensive infiltration and activation of neutrophils. Those active phases of chronic autoimmune inflammation are followed by the neutrophil prevalence, what for instance results with an expressive neutrophil accumulation in synovial fluids of the RA patients. In some autoimmune diseases, the formation of auto anti-bodies is expressed, leading to deposit formation of immune complexes of antigens and auto-antibodies in a body and a complementary system activation. Neutrophils penetrate into a tissue attempting to devour immune complexes, and infiltration and activation of neutrophils are exacerbated by an activated complement. An example of this type of disease is renal disease, especially the glomerulonephritis causing extensive kidney damage.

That is why the term "non-infectious inflammatory disease with neutrophil prevalence" includes, but is not limited to, chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS), bronchitis, bronchiectasis, emphysema, cystic fibrosis, inflammatory intestinal disease, gout arthritis, autoimune diseases characterised by acute phases with neutrophil prevalence as rheumatoid arthritis, autoimune diseases where infiltration of neutrophils is exacerbated by a complement activation as glomerulonephritis, and skin diseases, especially all kinds of neutrophil dermatoses including psoriasoforming dermatoses, like psoriasis and Reiter's syndrome, autoimmune bullous dermatoses, neutrophil dermatoses based on blood vessels, like leukocytoclastic vasculitis, Sweet's syndrome, pustular vasculitis, erythema nodosum and familiar Mediterranean fever, and pyoderma gangrenosum.

The term "non-infectious inflammatory disease with neutrophil prevalence" includes also all side effects, disorders or conditions caused by non-infectious inflammatory disease with neutrophil prevalence which can effect to other tissues or organs in a body except those which are effected by the inflammatory disease itself. Such examples are extraintestinal diseases like uveitis and chronic hepatitis which can be the consequence of inflammatory intestinal disease.

The term "active ingredient" or "active remedy" refers to all substances that effect to or recognise biological cells or their parts, especially cell organelles or cell components. Such active ingredients or remedies are of chemical origin. In particular, such active ingredients or remedies are diagnostic or therapeutic agents. In the context of present invention the term "active ingredients" or "active remedies" especially refers to therapeutic agents, for instance the substances that can be administered as a prophylaxis or within the course of disease, damage or condition to organisms requiring that kind of treatment in order to prevent or reduce or eliminate disease, damage or condition, especially non-infectious inflammatory disease with neutrophil prevalence.

In the context of present invention the term "treatment" refers to prophylactic and/or therapeutic activity of a drug or medicament that are defined as a pharmaceutical composition that includes pharmaceutical or diagnostic active compound in a combination with at least one additive like carrier.

"Azithromycin" refers to the macrolide compound N-methyl-11-aza-10-deoxo-10-dihydroerithromycin A (9-deoxo-9a-methyl-9a-aza-9a-homoerithromycin A) with an azalactone having the 15 member ring that can be obtained by Beckmann rearrangement of erythromycin A-oxime with subsequent Eschweiler-Clark reductive N-methylation, as it is basically described in US patent 4,517,359, US patent 4,328,334 and BE 892,357, incorporating completely the subject-matter of invention of these documents, regarding the methods of azithromycin production, in the subject-matter of the invention of the present patent application.

The term "pharmaceutically acceptable derivative" refers to non-toxic functional equivalents or derivatives of azithromycin that can be obtained by the replacement of atoms or molecular groups or bonds of azithromycin molecule, what does not change the basic structure of azithromycin, and which are distinguished from the structure of azithromycin at least at one position. The term "pharmaceutically acceptable derivative" includes for instance O-methyl derivatives of azithromycin that can be obtained as it is basically described in US patent 5,250,518, incorporating completely the subject-matter of invention of this document regarding the methods of O-methyl derivatives production in the subject-matter of the invention of the present patent application.

The term "pharmaceutically acceptable derivative" also includes esters of azithromycin that contain, after the hydrolysis of the ester bond, biological activity and the characteristics of azithromycin, and they are not biologically or by no other means undesirable. The techniques for preparation of pharmaceutically acceptable esters are for instance revealed in *March Advanced Organic Chemistry, 3rd. Ed., John Wiley & Sons, New York (1985) p. 1152.* Pharmaceutically acceptable esters useful as prodrugs are revealed in *Bundgaard, H., ed., (1985) Design of Prodrugs, Elsevier Science Publisher, Amsterdam.*

The term "pharmaceutically acceptable hydrate" refers to non-toxic solid or liquid compounds of azithromycin that keep biological activity of azithromycin and they are formed in the process of hydration where one or more molecules of water are attached to the molecule of azithromycin due to dipole forces. The term includes for instance mono- and dehydrates of azithromycin.

The term "pharmaceutically acceptable salts" refers to non-toxic alkali metals, alkaline earth metals and usually used ammonium salts including ammonium, barium, calcium, lithium, magnesium, potassium, protamine zinc salts, and sodium that are prepared by the methods known in the art. The term also includes non-toxic, e.g. pharmaceutically acceptable addition salts of acids that are usually prepared in the reaction of azithromycin and suitable organic or inorganic acid, like acetate, benzoate, bisulphate, borate, citrate, fumarate, hydrobromide, hydrochloride, lactate, laurate, maleate, napsylate, oleate, oxalate, phosphate, succinate, sulphate, tartrate, tosylate, valerate etc.

The term "pharmaceutically acceptable salts of acids" refers to salts that preserve the biological activity and the characteristics of free bases and that are not biologically or by no other means undesirable, formed from inorganic acids like hydrobromide acid, hydrochloride acid, nitric acid, phosphoric acid, sulphuric acid, and organic acids like acetic acid, benzoic acid, cinnamic acid, citric acid, ethanesulphonic acid, fumaric acid, glycolic acid, maleic acid, malic acid, mandelic acid, methanesulfonic acid, oxalic acid, propionic acid, p-toluenesulfonic acid, pyruvic acid, salicylic acid, succinic acid or tartaric acid.

The salts of the present invention can be obtained by dissolution of azithromycin in water or other suitable solvents with appropriate base and subsequent isolation of obtained salt of this invention by evaporation of solution, freezing and freeze-drying or by addition of some other solvent, for example, diethyl ether, in water and/or water/alcohol solution of azithromycin salts including the separation of insoluble crude salt. For the preparation of basic salts of azithromycin it is more convenient to use carbonates of alkali metals or hydrogen carbonates . The prepared salts are easily soluble in water.

The term "pharmaceutically acceptable complex or chelate" refers to non-toxic complexes or chelates of azithromycin with bivalent or trivalent metals that usually can be obtained as described in US patent 5,498,699, incorporating completely the subject-matter of invention of this document regarding the methods of azithromycin complexes or chelates production in the subject-matter of the invention of the present patent application. As the metals that form complexes or chelates the metals of II and III group that can form physiologically tolerable compounds can be used, especially Mg^{2+}, Al^{3+}, Fe^{3+}, Rh^{3+}, La^{3+} and Bi^{3+}. The azithromycin to metal ratio is preferably within the range from 1:1 to 1:4. In order to obtain complexes or chelates of azithromycin, the antibiotic reacts in the form of free base or salt, especially as hydrochloride, with a salt of bivalent and/or trivalent metal in the ratio 2:1 at the room temperature in a water solution or in the water/alcohol mixture at 8,0 to 11,0 pH with a metal hydroxide and/or carbonate, subsalicylate or with gel thereof. The preferred examples include chelates of azithromycin with antacids selected from the group of salts containing Al, Mg, Bi, chelates of azithromycin with surfactant and chelates of azithromycin with bismuth subsalicylate being in the gel form.

The term "pharmaceutically acceptable carrier" refers to carrying medium that does not disturb effect of biological activity of active ingredients and which is non-toxic for a host or a patient.

The active ingredient that is selected from the group consisting of azithromycin, the pharmaceutically acceptable derivative thereof, the pharmaceutically acceptable hydrate thereof, the pharmaceutically acceptable complexes or chelates thereof, and the pharmaceutically acceptable salt thereof, can also be administered to animals including the mammals, like rodents, primates, including humans, for the prevention or reduction or elimination non-infectious inflammatory disease with neutrophil prevalence. Thus, the present invention comprises the methods of therapeutic treatment of such disorders and diseases that include the application of the active ingredient of the present invention in an amount sufficient for achieving desirable activity of azithromycin *in vivo*. For example, the active remedy or ingredient of this invention may be used in

therapeutically or pharmaceutically effective amount for the treatment of various, including but not being limited to, COPD, ARDS and neutrophil dermatoses.

"Therapeutically or pharmaceutically effective quantity" applied to azithromycin or the compounds and compositions of the present invention that comprise azithromycin, refers to the amount of the compound or the composition that is sufficient to cause desirable biological result. That result can be a palliation of signs, symptoms or the causes of diseases, or any other desirable change of biological system. In this invention, the result will for instance include in specially preferred form the prevention, the elimination and/or the reduction of the symptoms or the causes of non-infectious inflammatory condition with neutrophil prevalence with an acute effect to granular enzymes of neutrophils, oxidising bang, oxidative protective mechanisms and neutrophil chemokine and circulating IL-1, IL-6, IL-8, as well as delayed effect to apoptosis of neutrophils and soluble adhesive molecules. In the preferred form, the active ingredients of present invention will be applied as a prophylaxis prior the eruption of non-infectious inflammatory disease with neutrophil prevalence.

In accordance with that, this invention also provides pharmaceutical compositions, including as the active ingredient azithromycin, the pharmaceutically acceptable derivative thereof, the pharmaceutically acceptable hydrate thereof, the pharmaceutically acceptable complex and chelate thereof, and the pharmaceutically acceptable salt together with the pharmaceutical carrier and the solvent. The compositions of this invention can be administered systemically or locally, especially by intravascular, oral, pulmonary, parenteral, e.g. intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection or inhalation, e.g. by fine powder formulation, transdermal, nasal, vaginal, rectal, or sublingual rout of administration. An active remedy or ingredient is preferably given in a pharmaceutically effective amount.

Solid dosage forms for oral administration include capsules, lingualets, tablets, pills, powders, liposomes, patches, film tablets with sustained release and granules. In such solid oral forms, an active ingredient is mixed with at least one inert pharmaceutically acceptable carrier like lactose, sucrose, or starch. Such dosage units can also include additional substances besides inert solvents, e.g. sliding agents like magnesium stearate. Considering capsules, tablets and pills the dosage forms can also include the volume enlarging agents and/or buffers, as well as flavours. Besides, tablets and pills can be prepared with an enteric coating.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspension, syrups, with elixirs containing inert diluents usually used in the art like water. Beside such inert diluents, the formulations can also contain adjuvants like salts for osmolal pressure variation, pH adjusting agents, skin penetrating agents, moisturisers, emulsifying agents, suspending agents, sweeteners, flavours and odours.

The pharmaceutical compositions according to this invention for parenteral administration include sterile water and non-water solutions, suspensions and emulsions. The examples of non-water solutions or carriers are propylene glycol, polyethylene glycol, vegetable oils, like olive oil, corn oil, gelatine, and injectable organic esters, like ethyl oleate. Such dosage units can also contain excipients as preservatives, moisturisers, emulsifying agents, and dispersing agents. They can be sterilised, for example by filtration through the filter that retains bacteria, by including sterilising agents into compositions, by radiating the compositions, or by hitting the compositions. They can also be produced using sterile water, or some other sterile injectable medium, directly before the use.

The injecting formulations will include a physiologically acceptable medium as water, saline, PBS, ethanol diluted with water, ethylene glycol diluted with water, and the like. The water soluble preservatives sodium bisulphite, sodium thiosulphate, ascorbate, benzalkonium chloride, chlorobutanol, thiomersal, phenylmercuric borate, p-hydroxybenzoates, benzyl alcohol and phenyl-ethanol. Those agents can be present individually in the amounts ranging from about 0.001 to about 5% by weight, preferably from about 0.01 to 2%. The suitable water soluble buffers that can be used are carbonates of alkali or alkaline earth metals, phosphates, bicarbonates, citrates, borates, acetates, succinates and the like, as sodium phosphate, citrate, borate, acetate, bicarbonate, and carbonate. The additives like carbo-methyl cellulose can be used as the carrier in the amounts ranging from about 0.01 to 5% by weight. The formulation can vary depending on the purpose of the formulation, on the specific route of disease treating, on the intended treating etc.

The compositions for rectal or vaginal administration are preferably suppositories that can contain, beside an active ingredient, excipients like cacao butter, or suppository wax. The compositions for nasal or sublingual administration are also prepared using the standard excipients well known in the art.

The compositions that contain an active remedy or ingredient of the present invention can be used for prophylactic and/or therapeutic treatment. In therapeutic application, the compositions are administered to the patients already suffering from a disease, as described above, in an amount sufficient for a treatment or for at least partial palliation of disease symptoms and its complications, e.g. in a therapeutically sufficient amount.

In prophylactic application, the compositions containing an active remedy or ingredient of the present invention are administered to the patient who is susceptible to, or in some other manner in a danger from certain disease. That amount is defined as "prophylactically effective amount". At this usage, the precise amounts depend on patient's health condition and body weight.

The pharmaceutical compositions of this invention can be administered in a depot form as the compositions with sustained release. Such compositions with sustained release can include the particles of an active remedy or ingredient in matrix, for instance made of collagen.

The amounts of an active remedy or ingredient required for an effective therapy will depend upon many various factors, including the rout of administration, the target place, the physiological condition of a patient, and other applied medicaments.

An active remedy or ingredient of the present invention selected from the group consisting of azithromycin, the pharmaceutically acceptable derivative thereof, the pharmaceutically acceptable hydrate thereof, the pharmaceutically acceptable complex or chelate thereof, and the pharmaceutically acceptable salt thereof are effective in treating of non-infectious inflammatory diseases with neutrophil prevalence when applied in the amount ranging from about 10 mg to 2000 mg a day, preferably from about 30 to 1500 mg. The specific applied dose that is used depends on the special condition that is treated, the rout of administration, as well as the clinician evaluation depending on factors as the solemnity of the condition, the age and general condition of a patient.

Active remedies or ingredients of the present invention can be administered individually or in a combination with other medicaments that are momentary used for the treating of non-infectious inflammatory diseases with neutrophil prevalence like non-steroid anti-inflammatory agents as methyl xanthine antiinflammatory agents, steroid anti-inflammatory agents, immunomodulators, immunosuppressive agents, bronchodilators, anti-rheumatic agents, corticosteroids (β2-agonists, cholinergic antagonists, and the like), what can eventually diminish the latter for 50% or 25% due to anti-inflammatory activity of the active ingredients of this invention.

The composition, preferably the water soluble composition, of the present invention, can further contain water soluble protein that is injectable into body fluids without exposing any significant pharmacological activity in a concentration used in a dosage unit of the present invention (further "water soluble protein"). As such are preferred water soluble protein like albumin, globulin, collagen and/or gelatine in serum. That protein can be added in an amount that is usually used for injectable pharmaceutical compositions. Thus, for example, the weight ratio of water soluble protein and an active remedy or ingredient of the present invention is about 0.0001:1 to 100:1.preferably about 0.001:1 to about 10:1, or more preferably about 0.01 to about 1:1.

In addition, the invention also relates to above cited active remedies or ingredients themselves or compositions containing them, especially in a dried and/or pure form, or in water or water/alcohol solution. pH of solution prepared from the water soluble composition or active ingredient of the present invention must be so adjusted that mentioned pH has no negative effect to the activity of pharmacologically active peptide, and still being within general acceptable range for injections and further, it mast be so adjusted that said pH will neither lead to a great change of viscosity of solution nor will permit a formation of a precipitate and the like. The solution preferably has to have pH ranging from 4 do 7, more preferably 5 to 6, and the most preferably 5.3 to 5.5.

When water soluble composition of the invention turns into a ready for use water solution, the concentration of pharmacologically active remedy or ingredient or salt thereof in a said solution preferably has to be 0.0000001 to 10% (w/v), even better 0.000001 to 5% (w/v), or even more better about 0.00001 to 1% (w/v).

The composition of this invention preferably has to have a dosage unit form containing pharmacologically active remedy or ingredient of the invention and, if necessary, further additives as above mentioned water soluble protein. For example, two or three above mentioned compounds are prepared in ampoule or vial by dissolving or suspending in sterile water or sterile saline. In that case, the method of preparation can include

admixing of solution of pharmacologically active remedy or ingredient and furthermore, if necessary, solutions of additives or adding of additive in a powder form into a solution of pharmacologically active remedy or ingredient, or any other combination of suitable methods. A dosage unit can also be prepared by addition of sterile water or sterile saline into freeze-dried cake or powder dried in a vacuum where are together pharmacologically active remedy, and if necessary, an additive. That unit dosage form can comprise one or more common additives, like the pH corrective agents (e.g. glycine, hydrochloric acid, sodium hydroxide), local anesthetics (e.g. Xylocaine hydrochloride, chlorobutanol), isotonic agents (e.g. sodium chloride, mannitol, sorbitol), emulsifying agents, absorption inhibitors (e.g. Tween ® 60 or 80), talc, starch, lactose and tragacanth, magnesium stearate, glycerol, propylene glycol, preservatives, benzyl alcohol, methyl hydroxy-benzoate and/or hydrogenated peanuts oil. This unit dosage form can furthermore comprise pharmaceutically acceptable excipient as polyethylene glycol 400 or dextran.

The composition of this invention is made by admixing the compounds prepared according to the common method. The goal of admixing the ingredients of this invention is to preserve activity of pharmaceutically active remedy and to reduce to minimum the production of bubbles during the procedure. The ingredients are put into a vessel (for example a bottle or a cylinder) simultaneously or in some order.

The atmosphere in a vessel can be, for example, the sterile clean air or the sterile clean nitrogen. Obtained solution can be placed into small vials or ampoules, and can be further subjected to a freeze-drying.

The liquid form or the form of freeze-dried powder of the present invention can be dissolved or dispersed in a solution of a biodegradable polymer as the copolymer of poly(lactic-glycolic) acid, poly(hydrobutyric) acid, the copolymer of poly(hydrobutyric-glycolic) acid, or the mixture thereof, and then can be formulated, for example, into films, microcapsules (microspheres), or nano-capsules, especially in the form of soft or hard capsules.

Furthermore, the composition of this invention encapsulated into liposomes, including phospholipids, types of cholesterol, or derivatives thereof, can be further dispersed in the saline or hyaluronic acid dissolved in the saline.

A soft capsule can be filled with the liquid form of the composition of this invention. A hard capsule can be filled with freeze-dried powder of the present invention, or freeze-dried powder of the present invention can be compressed into tablets for rectal administration or oral administration.

Of course, the composition of this invention can be delivered into the filled injections for the single use.

Although only periphery forms of the invention are specifically described above, it is appreciated that the changes and the variations of the invention are possible without the collision regarding the spirit and attended scope of the invention. Further preferred forms of the present invention are described in the claims.

Examples

The study on normal volunteers has been conducted through, and the activity of azithromycin administered in a dosage of 3 × 500 mg has been observed regarding selected parameters relevant for the inflammation.

The administration of the drug, blood sample and plasma drawing

Each examinee received two standard 250 mg capsules of azithromycin (Sumamed®, PLIVA, Zagreb) in three subsequent days. Directly before treatment and 2h, 24h and 28 days after the third and the final dose of azithromycin, the blood was drowen from cubital vein into the tubes containing EDTA. The aliquots were taken for the cell counting, the preparation of the smear, the isolation of polymorphonuclear cells and serum.

The Analysis of principal azurophil granular enzymes

Leukocyte granules are the organelles, separated by the membrane, that contain larger number of anti-microbe proteins. Besides containing degradation enzymes that can be extracellularly secreted from neutrophils or can be released into phagocyte vesicles differently, the membranes of lot of kinds of these granules and vesicles contain important molecules as specified receptors (e.g. fMLP receptor) and cytochrome b NADPH oxydases.

a) The analysis of myeloperoxidase

The enzyme myeloperoxidase (MPO) is the protein having 135.000 dalton that contains two heavy and two light chains having 55.000 and 15.000 dalton MPO is placed into the principal or the azurophil granules of granulocyte cells. The function of MPO is to provide the reactive oxygen metabolites that are important for microbicide activity of neutrophils. The production of oxygen metabolites depends on components of MPO-negative granules (wherein flavocytochrome b_{558}, the important component of NADPH oxydase is placed) and components of azurophil MPO-positive granules. MPO transforms relatively harmless product of NADPH oxydase, H_2O_2 into hypochlorous acid. The biological significance of MPO activity in granulocytes is the strong anti-microbial activity depending on oxygen and being in conjunction with a mobilisation of all granules in inflammatory granulocytes within inflammatory process, especially after phagocyte stimulus caused by immune complexes.

The MPO activity was determined from the intensity of neutrophil coloration in blood smears and in cell lysates by ELISA. After the fixation in ethanol-formaldehyde, the smears were incubated in a substrate solution that contained hydrogen peroxide and benzidine (SIGMA). After incubation, the smears were contrast-dyed with Giemsa solution. The positiveness of 100 granulocytes MPO values was estimated and evaluated ranging from 0 to 4+ based on the intensity of precipitate colour in cytoplasm. That is why the evaluation grade can range from 0 to 400. The normal values of grade range (290-300) were taken from this study prior the administration of azithromycin. MPO activity was also estimated using digital smear image taken by digital camera with the strong amplification (× 1000)of light microscope. MPO neutrophil activities in blood smears were reduced from 2h to 24h after the final dose of azithromycin and returned to staring line after 28 days (Table 1). The MPO enzyme protein concentration determined by ELISA in neutrophil lysates is shown in T able 1. The change in neutrophil enzyme protein followed the same pattern of changes that was present at the activity of intracellular enzyme, being reduced from 2h to 24h after the final dose of azithromycin and returning to the starting values after 28 days. The both methodological approaches of MPO determination confirmed each other. The degranulation that was manifested by lower density of MPO neutrophils, and that was determined cytochemically, was connected with lower ELISA MPO concentrations in neutrophil lysates.

b) The analysis of N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase

Glycosidases are the enzymes that catalyse hydrolysis of glucoside bonds of oligosaccharides and other glucosides. They are specific for the glucoside part of substrate molecule. N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase are such kind of enzymes. They are lysosomal enzymes, both being placed into azurophil (principal; peroxidase-positive) neutrophil granules. As the degranulation of neutrophils is present during an inflammation, many of authors chose these enzymes as the markers of degranulation and for estimation of neutrophil reactivity. The catalytic concentration of both enzymes in serum and in neutrophil lysates was determined by use of fluorometric method described by O'Brien et al., *(New Engl. J. Med. (1970) 283: 15-20)* for NAGA and Glaser & Sly *(J. Lab. Clin. Med. (1973) 82: 969)* for β-glucuronidase.

The results showed (Table 1) that NAGA activity in serum increased for about 30% two hours after the final dose. 24 hours after the final dose it was about 50% higher than the starting values. 28 days after that, NAGA values in serum were still 70% higher than the starting values. The increasing of NAGA in serum was followed by the reduction of enzymes activity in PMN. 2 hours after the final dose, the decreasing of about 70% of NAGA in granulocytes was determined. 24 hours after that, the NAGA activity in PMN increased for about 30%, but it still was about 40% lower in a comparison with the starting values. 28 days after that, the NAGA activity increased for about 40% in a comparison with the starting values (Table 1).

The β-glucuronidase activity in serum showed no changes during the first 24 hours after the final dose. 28 days later, the values in serum were 40% higher than the starting values. The β-glucuronidase activity in PMN decreased for about 20% after two hours, and for about 50% 24 hors later in a comparison with the starting values. Nevertheless, 28 days after that, the β-glucuronidase activity in PMN was much higher (about 300%) in a comparison with the starting values (Table 1).

When the activity of glucosides is analysed, it is apparent that azithromycin administered to normal volunteers leeds to 40-50% release of enzymes from azurophil granules within 24 hours after the final dose. The decreasing of NAGA activity in PMN is followed by the increasing of NAGA activity in serum. The activity of these two enzymes in serum showed slow increasing above the base line (before azithromycin administration) 2h and 24h after the final drug dose, being further increased 28 days later (Table 1).

Summarised, the enzymes that were released from principal azurophil granules of neutrophils showed tendency of presence in serum with slightly higher activities 2–24h after the administration of azithromycin, while during the same period, their activities were lower in neutrophils of periphery blood, what pointed that they were released by the granulation. NAGA was released quickly after the administration of azithromycin, while MPO and β-glucuronidase showed sustained release. The recovery of these enzymes varied too.

The research of neutrophil oxidising bang

All aerobe organisms use oxygen for energy production. But, there are lots of indication that the advantages of oxygen usage are connected with a risk that the oxidative process can cause damages too. During phagocytosis when neutrophils are stimulated, they are conducted to oxidising bang, with a production and release of reactive oxygen radicals. These reactive oxygen radicals function as the principal mechanisms being used by phagocytes for mediation of their anti-microbe activity. The reactions are characterised by rapid intake (consumption) of oxygen with a subsequent reduction of oxygen to superoxide (O^{2-}). This is catalysed by NADPH oxidase using NADPH or NADH like an electron donor. When these defence mechanisms are improperly directed, it leads to the tissue damage.

a) Determination of chemoluminescence development
The production of free oxygen radicals caused by activated cells is often determined by chemoluminescence measurement (CL). The produced oxygen radicals react with a substance that produces photons (e.g. Luminol), and the resulting light emission is measured by a photocell. The chemoluminescence can be discovered as the result of stimulation (e.g. fMLP) of leukocytes and it is the measure of their oxidative cytotoxic activity (*Allen et al., Biochem. Biophys. Res. Commun. (1972), 47: 679*). The results of that study, given in Table 1, show that azithromycin inhibits chemoluminescence inception of stimulated neutrophils that are isolated from the blood of the people being treated with azithromycin.

b) System for determination of cytochrome c
Neutrophils were incubated with cytochrome c and stimulated with fMLP (*Cohen and Chovaniec, (1987), J. Clin. Invest. 61: 1081-1087*). The absorbencies were recorded at 550 nm and 540 nm and the results were expressed as delta A. Neutrophil oxidising bang, as the response to bacterial peptide fMLP was inhibited by dosage of azithromycin that was lasting for three days (Table 1). Using cytochrome c and luminol as a determination system, the inhibition was discovered already 2h after the final dose of azithromycin, and it was greater after 24h, and it did not return to the normal level 28 days later.

Consequently, azithromycin should be considered as inhibitor of oxidising bang. Thus, azithromycin provides the base for various diseases where the production of radicals in neutrophils (oxidising bang) becomes too excessive, like COPD.

Analysis of glutation peroxidase and glutation reductase

The oxygen radicals and lipid peroxidases are involved in the pathogenesis of many diseases. The biological activity of free radicals is controlled *in vitro* by wide range of anti-oxidants, like α-tocopherol (Vitamin E), ascorbic acid (Vitamin C), β-carotene, reduced glutation (GSH), and anti-oxidative enzymes (superoxide dismutase, SOD, glutation peroxidase GSHPx, catalase, CAT) (*Benabdeslam et al., Clin. Chem. Lab. Med. (1999) 37: 511-516; Mates et al., Blood Cells Mol. (1999), 25: 103-109*). Recently, anti-oxidation function were definitely brought into correlation with anti-inflammatory and/or immunosuppressive characteristics (*Mates et al., Blood Cells Mol. (1999), 25: 103-109*). The production of free radicals and disturbance of redox status can modulate the expression of various inflammatory molecules (*Sundaresan et al., Science (1995), 270: 296-299; Kaouass et al., Endocrine (1997), 6: 187-194*) that have an influence on certain cell processes that amplify the inflammation and cause the tissue damage (*Tsai et al., FEBS Lett. (1997), 436: 411-414*).

The sell glutation peroxidase (GSHPx) is a tetrameric protein where each of four identical sub-units contains one atom of selene (Se) in a form of selenocysteine on the active site (*Misso et al., J. Leukoc. Biol. (1998), 63: 124-130*). GSHPx has a role in a detoxification of H_2O_2 and turns lipid hyperoxides into non-toxic alcohols (*Akkus et al., Clin. Chim. Acta (1996), 244: 221-227; Urban et al., Biomed. & Pharmacoter. (1997), 51: 388-390*). In this study on normal volunteers that were treated with azithromycin, the activity alterations of intracellular GSHPx polymorphonuclear were determined using the commercial kit RANSEL (Randox Laboratories). GSHPx catalyses the oxidation of gluatation with cumene hydroperoxide. In a presence of

glutation reductase and NADPH, oxidised glutation is immediately turned into reduced form with a simultaneous oxidation of NADPH to $NADP^+$. An absorbance reduction was measured at 340nm.

Glutation reductase is the enzyme being present everywhere that catalyses the reduction of oxidised glutation (GSSG) to glutation (GSH). Glutation reductase is important for glutation redox cycle that maintains certain levels of reduced cell GSH. GSH is antioxidant that reacts with free radicals and organic peroxides, in an amino acid transmission, and as the substrate for GSHPx and glutation S-transferases in a detoxification of organic peroxides and a metabolism of xenobiotics. Glutation reductase was determined using BIOXYTECH® GR-340™ colorimetric test for glutation reductase (OXIS International, Inc.). Shortly, the oxidation NADPH to $NADP^+$ is catalysed by the limiting concentration of glutation reductase.

GSHPx activity in neutrophil lysates (expressed per g of protein) was not changed 2h after the final dose of azithromycin, but it decreased significantly 24h after that final dose (Table 1). The activity turned back to the starting values 28 days after that. The activity of glutation reductase in cell lysates (expressed per g of protein) showed similar tendency, decreasing significantly 2h and 24h after final dose of azithromycin, and coming back to the normal values and reaching the levels higher than normal 28 days after the treatment (Table 1).

The analysis of apoptosis

The administration of azithromycin that was lasting for three days had delayed pro-apoptotic effect to granulocytes that was shown by the morphology of blood smear. The results are given in Table 1. The number of counted apoptotic cells was being increased constantly after the dosage of azithromycin that was lasting for three days, achieving the statistical significance 28^{th} day after the final dose. The increased number of apoptotic cells indicates to the decreased number of active, potentially damaging neutrophils.

The analysis of cytokine and chemokine

The other acute, but potentially antiinflammatory activities of azithromycin were also discovered in this invention.

Interleukin-8, the member of chemokines CXC sub-group, specific for neutrophils, is the strong chemotactic and activating factor for neutrophils (*Oppenheim, J. Am. Rev. Immunol. (1999), 9:617*). It binds to at least two G protein bounded receptors (IL-8R1and IL-8R2). These receptors are functionally different. The responses, as the changes in cytosol concentrations of Ca^{2+} ions and the release of granule enzymes, are mediated by both receptors, while oxidising bang and phospholipase D activation exclusively depend on stimulation of IL-8R1 (*Johnes et al., Proc. Natl., Acad. Sci. USA (1996), 93: 6682-6686*). IL-8 is the key mediator in circulating neutrophil recruitment. This chemokine is expressed as the response to inflammatory stimuli, and is secreted by different types of the cells, including lymphocytes, epithelial cells, keratinocytes, fibroblasts, endothelial cells, the smooth muscle cells and neutrophils. In this last case, IL-8 is one of the most secreted (ant the most extensively studied) cytokines that are produced by neutrophils. Interestingly, the neutrophils represent the principal cell target for IL-8, to which they respond with chemotaxis, the granule content release, the oxidising bang, the increased expression, and the receptor activity on cell surface (up-regulation), the increased adhesion to non-stimulated endothelial cells, and the transmigration through endothelium. The substances that can stimulate the production of IL-8 in human neutrophils are: TNF-α, IL-1β, GM-CFS, leukotriene B4, PAF, fMLP, lactoferrin, LPS and many others (*Cassatella, M.A., Adv. Immunol. (1999), 73: 369-509*). IL-8 retards the spontaneous and TNF-α mediated apoptosis of human neutrophils (Ketritz et al., Kidney Int. (1998), 53: 84-91). IL-8 is predominant C-X-C chemokine and dominant chemoattractant of neutrophils that is accumulated in supernatant of LPS-stimulated human alveolar macrophages (*Goodman et al., Am. J. Physiol. (1998), 275: L87-L95*).

It is reported that erythromycin has got the inhibitory activity to expression of IL-8 in human epithelial cell, and that kind of activity is probably relevant for its clinical effects (*Takizawa et al., Am. J. Respir. Crit. Care Med. (1997), 156: 266-271*).

Roxythromycin has also the ability of IL-8 production reduction in fibroblasts of nasal polyps (*Nonaka et al., Acta Otolaryngol. (1998) Suppl. 539: 71-75*). In synoviocyte in rheumatoid arthritis, the production of IL-1α, IL-6, IL-8, GM-CSF can be inhibited by clarithromycin (*Matsuoka et al., Clin. Exp. Immunol. (1996), 104(3): 501-8*). *Ex vivo* determination of IL-8 production in entire blood also confirmed the potential of erythromycin for inhibition of IL-8 production (*Schultz et al., J. Antimicrob. Chemother. (2000), 46: 235-240*). Recently, the

similar fact was reported for bronchial epithelial cells (*Deski M. et al., Biochim. Biophys. Res. Commun. (2000), 267: 124-128*). However, in recent study it was reported about of non-existence of azithromycin modulatory activity to the production of IL-8 PMN-a *in vitro* (*Koch et al., J.Antimicrob. Chemother. (2000), 46: 19-26*).

The concentrations of cytokines and chemokines were determined using ELISA kit. After the administration of azithromycin that was lasting for three days, several different response patterns of cytokines and chemokines concentrations in serum were observed. Rapid and significant concentrations reduction in chemokine plasma that stimulates neutrophils, IL-8, GRO-α was observed 2h and 24h after the final dose of azithromycin (Table 1). The IL-8 concentration basically returned to starting values after 28 days, while in the same time GRO-α was reduced.

These results clearly indicate the acute inhibitory activity of azithromycin to the release of IL-8 *ex vivo*, and that characteristic is spread to inhibition of chemokine GRO-α release. However, it must be pointed out that the concentration of chemokine in serum was measured. Because of that we cannot deduce any conclusions regarding the chemokine cell(s) resources.

The low basic concentration of IL-1 in serum gradually increased after the final dose of azithromycin, achieving statistically significant increase after 24h (Table 1). The concentration returned to the starting values after 28 days of azithromycin administration. In spite of it, the concentration of IL-6 in serum showed significant reduction achieving statistical significance after 28 days of last dose of azithromycin (Table 1).

The analysis of adhesive molecules

In a contrary to previously revealed data (*Seman et al., J. Cardiovasc. Pharmacol. (2000), 36: 533-537*) wherein the treatment with azithromycin did not significantly influence to the levels of soluble VCAM in plasma, in this research the sVCAM reduction in serum was observed 24h after the final dose of azithromycin, staying significantly reduced after 28 days, that indicates that azithromycin has got the inhibitory potential in both producing of neutrophil chemotactic peptides and expression and release of adhesive molecules for activated leukocytes (Table 1). For the quantitative determination of human sVCAM concentration in serum ELISA kit was used (R&D Systems, UK).

The proteins in the samples PMN were determined by Bradford's method (Anal. Biochem. (1976) 72: 248-254) using the bovine albumin in serum as the standard.

Table 1	UNITS	Starting Values	2h and 30 min	24h	28 days
DEGRANULATION					
Myeloperoxidase (evaluation)		337±29	326 ±26	315±22*	347±18
Myeloperoxidase (coloration)		105±13	130±16*	131±17*	115±19
Myeloperoxidase (PMN)	µg/ mg protein	54.22±12.61	70.85±19.91	26.74±2.51*	70.01±17.62
NAGA (PMN)	nmol 10^{-6} cells × min^{-1}	4.15±01.6	1.13±0.72*	2.62±1.6*	5.95±3.7
β-glucuronidase (PMN)	nmol 10^{-6} cells × min^{-1}	4.12±2.7	3.21±2.3	1.58±0.4*	15.37±11.4*
NAGA (serum)	µmol L^{-1} × min^{-1}	9.16±1.6	11.52±2.2	13.7±1.5*	14.87±1.9*
β-glucuronidase (serum)	µmol L^{-1} × min^{-1}	2.88±0.7	3.01±0.6	2.95±0.5	3.93±1.2*
CYTOKINES (serum)					
IL-1	pg/mL	0.291±0.11	0.533±0.15	1.07±0.19*	0.29±0.20
IL-6	pg/mL	3.4±1.05	2.7±1.49	2.5±1.48	1.15±0.61*
CHEMOKINES (serum)					
IL-8	pg/mL	29.47±15.44	10.61±3.81*	14.60±10.75*	23.03±19.72
GRO-α	pg/mL	124.1±33.02	109.6±30.35*	107.9±27.83*	90.4±2.02*
APOPTOSIS (leukocytes)	Apoptotic cells/1000 leukocytes	0.333±0.655	0.833±1.029	1.417±1.240	2.583±2.02*
ADHESION MOLECULES					
sV-CAM	ng/mL	13.59±2.90	12.21±4.12	10.29±2.12*	10.74±2.05*
OXIDISING BANG (PMN)					
fMLP-luminol	A.U.	29335±1957	14774±1175*	5053±3804*	9879±13880*
fMLP- cytochrome C	ΔA	0.020±0.014	0.007±0.015*	-0.018±0.010*	-0.0011±0.0010*
GLUTATION PEROXIDASE (PMN)	mU/10^6 PMN	5.32±2.0	5.3±2.9	1.6±1.3*	8.0±5.2
GLUTATION REDUCTASE (PMN)	mU/10^6 PMN	9.63±1.16	7.39±1.23*	7.91±0.87*	11.27±2.24*

*$p<0.01$ in a comparison with the starting values (Wilcoxon).

CLAIMS

1. Usage of the active compound chosen from a group consisting of azithromycin, its pharmaceutically acceptable derivative, its pharmaceutically acceptable hydrate, its pharmaceutically acceptable complex or chelate, and its pharmaceutically acceptable salt, for preparing pharmaceutical compositions for treating noninfectious inflammatory diseases with neutrophil prevalence in people and animals.

2. Usage according to Claim 1, **characterised in that** the noninfectious inflammatory disease with neutrophil prevalence is a pulmonary disease, including a chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS), bronchitis, bronchiectasis, cystic fibrosis and emphysema.

3. Usage according to Claim 1, **characterised in that** the noninfectious inflammatory disease with neutrophil prevalence is a skin disease, especially a neutrophil dermatoses including psoriasoforming dermatoses, like psoriasis and Reiter's syndrome, autoimmune bullous dermatoses, neutrophil dermatoses based on blood vessels, like leucocytoclastic vasculitis, Sweet's syndrome, pustular vasculitis, erythema nodosum and familiar Mediterranean fever, and pyoderma gangrenosum.

4. Usage according to Claim 1, **characterised in that** the noninfectious inflammatory disease with neutrophil prevalence is an autoimmune disease where neutrophil infiltration is diminished by the complement activation, especially kidney diseases, including glomerulonephritis.

5. Usage according to Claim 1, **characterised in that** the noninfectious inflammatory disease with neutrophil prevalence is an intestinal disease, including an inflammatory intestinal disease.

6. Usage according to Claim 1, **characterised in that** the noninfectious inflammatory disease with neutrophil prevalence is an autoimmune disease characterised by acute phases with neutrophil prevalence, like a rheumatoid arthritis.

7. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an azithromycin *O*-methyl-derivative.

8. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an azithromycin ester.

9. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an azithromycin monohydrate.

10. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an azithromycin dihydrate.

11. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an azithromycin complex or chelate with metal ions.

12. Usage according to Claim 11, **characterised in that** the ratio of azithromycin to metal is 1:1 to 1:4.

13. Usage according to Claim 11 or 12, **characterised in that** metal ions are bivalent metal ions.

14. Usage according to Claim 11 or 12, **characterised in that** metal ions are trivalent metal ions.

15. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an alkali metal, alkaline earth metal or azithromycin ammonium salt.

16. Usage according to any of Claims from 1 to 6, **characterised in that** the active ingredient is an azithromycin addition salt with acids.

17. Usage according to Claim 16, **characterised in that** the acid addition salt is prepared with an inorganic acid.

18. Usage according to Claim 16 or 17, **characterised in that** the inorganic acid is a hydrobromic acid, nitric acid, phosphoric acid or sulphuric acid.

19. Usage according to Claim 16, **characterised in that** the acid addition salt is prepared with an organic acid.

20. Usage according to Claim 19, **characterised in that** the organic acid is an acetic acid, benzoic acid, cinnamic acid, citric acid, ethanesulphonic acid, fumaric acid, glycolic acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulphonic acid, oxalic acid, *p*-toluenesulphonic acid, piruvic acid, salicylic acid, succinic acid or tartaric acid.

21. Usage according to any of Claims from 1 to 20, **characterised in that** the pharmaceutical composition contains the active ingredient in a sufficient amount to abolish or diminish the disease, or to stop its progression.

22. Usage according to Claim 21, **characterised in that** pharmaceutical compositions are applied once to thrice a day in doses from 10 mg to 2000 mg of the active ingredient.

23. Usage according to Claim 22, **characterised in that** pharmaceutical compositions are applied once to thrice a day in doses from 30 mg to 1500 mg of the active ingredient.

24. Usage according to any of Claims from 1 to 23, **characterised in that** pharmaceutical compositions are applied orally in solid or liquid dosing forms.

25. Usage according to Claim 24, **characterised in that** solid pharmaceutical compositions for the oral application are capsules, lingualettes, tablets, pills, powders, liposomes, plasters, envelopes with the prolonged effect, and granules.

26. Usage according to Claim 24 or 25, **characterised in that** solid pharmaceutical compositions for the oral application contain at least one inert pharmaceutically acceptable carrier.

27. Usage according to Claim 26, **characterised in that** inert pharmaceutical carriers are lactose, sucrose or starch.

28. Usage according to any of Claims from 24 to 27, **characterised in that** solid pharmaceutical compositions for the oral application include additional substances chosen from a group consisting of sliding agents like the magnesium stearate, agents for volume enlargement and/or buffers and flavours.

29. Usage according to any of Claims from 24 to 28, **characterised in that** solid pharmaceutical compositions for the oral application are prepared with enteric envelopes.

30. Usage according to Claim 24, **characterised in that** liquid pharmaceutical compositions for the oral application are pharmaceutically acceptable emulsions, solutions, suspensions or syrups.

31. Usage according to Claim 30, **characterised in that** the liquid pharmaceutical composition for the oral application contains at least one inert pharmaceutically acceptable carrier.

32. Usage according to Claim 31, **characterised in that** the inert pharmaceutically acceptable carrier is a water or saline.

33. Usage according to any of Claims from 30 to 32, **characterised in that** the liquid pharmaceutical composition for the oral application contains additional substances, chosen from a group consisting of excipients, salts for the osmotic pressure varying, agents for the pH adjustment, agents for the skin penetration, wetting agents, emulsifiers, and suspending agents.

34. Usage according to any of Claims from 1 to 23, **characterised in that** pharmaceutical compositions are applied parenterally.

35. Usage according to Claim 34, **characterised in that** pharmaceutical compositions for the parenteral application are infusions or injections.

36. Usage according to Claim 34 or 35, **characterised in that** pharmaceutical compositions for the parenteral application are sterile water or non-water solutions, suspensions or emulsions.

37. Usage according to any of Claims from 34 to 36, **characterised in that** pharmaceutical compositions for the parenteral application include non-water solvents or carriers.

38. Usage according to Claim 37, **characterised in that** non-water solvents or carriers are propylene glycol, polyethylene glycol, vegetable oils like olive oil and corn oil, gelatine, and injectable organic esters like ethyl oleate.

39. Usage according to any of Claims from 34 to 38, **characterised in that** pharmaceutical compositions for the parenteral application include auxiliaries like maintaining agents, wetting agents, emulsifiers and dispersing agents.

40. Usage according to any of Claims from 1 to 23, **characterised in that** pharmaceutical compositions are applied rectally or vaginally.

41. Usage according to Claim 40, **characterised in that** pharmaceutical compositions for the rectal or vaginal application are suppositories, enemas or foams.

42. Usage according to Claim 40 or 41, **characterised in that** pharmaceutical compositions for the rectal or vaginal application contain excipients like a cocoa butter or wax for suppositories.

43. Usage according to any of Claims from 1 to 42, **characterised in that** pharmaceutical compositions for treating noninfectious inflammatory diseases with neutrophil prevalence contain one or more additional active ingredients useful for the treatment of such diseases, chosen from a group consisting of non-steroid anti-inflammatory agents, steroid anti-inflammatory agents, bronchodilators, antirheumatic agents, immunomodulators, immunosuppressive agents, corticosteroids, β2-agonists and cholinergic antagonists.

44. Usage according to Claim 43, **characterised in that** the dose of the additional active ingredient is decreased compared to the pharmaceutical composition containing solely one additional active ingredient.

45. Pharmaceutical composition for treating noninfectious inflammatory diseases with neutrophil prevalence in people and animals, which includes azithromycin as the active ingredient, its pharmaceutically acceptable derivative, its pharmaceutically acceptable hydrate, its pharmaceutically acceptable complex or chelate, and its pharmaceutically acceptable salt.

46. Pharmaceutical composition according to Claim 45, **characterised in that** the active ingredient is an azithromycin *O*-methyl-derivative or azithromycin ester.

47. Pharmaceutical composition according to Claim 45, **characterised in that** the active ingredient is an azithromycin monohydrate or dihydrate.

48. Pharmaceutical composition according to Claim 45, **characterised in that** the active ingredient is an azithromycin complex or chelate with bivalent or trivalent metal ions.

49. Pharmaceutical composition according to Claim 48, **characterised in that** the ratio of azithromycin to metal ions is 1:1 to 1:4.

50. Pharmaceutical composition according to Claim 45, **characterised in that** the active ingredient is an alkali metal, alkaline earth metal or azithromycin ammonium salt.

51. Pharmaceutical composition according to Claim 45, **characterised in that** the active ingredient is an azithromycin acid addition salt.

52. Pharmaceutical composition according to Claim 51, **characterised in that** the acid addition salt is prepared with an inorganic acid, like a hydrobromic acid, nitric acid, phosphoric acid or sulphuric acid.

53. Pharmaceutical composition according to Claim 51, **characterised in that** the addition salt obtained by the acid addition is prepared with an organic acid, like an acetic acid, benzoic acid, cinnamic acid, citric acid, ethanesulphonic acid, fumaric acid, glycolic acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulphonic acid, oxalic acid, *p*-toluenesulphonic acid, piruvic acid, salicylic acid, succinic acid or tartaric acid.

54. Pharmaceutical composition according to any of Claims from 45 to 53, **characterised in that** the active ingredient is contained in a sufficient amount to abolish or diminish the disease, or to stop its progression.

55. Pharmaceutical composition according to any of Claims from 45 to 54, **characterised in that** it includes one or more additional active ingredients useful for the treatment of such diseases, chosen from a group consisting of non-steroid anti-inflammatory agents, steroid anti-inflammatory agents, bronchodilators, antirheumatic agents, immunomodulators, immunosuppressive agents, corticosteroids, β2-agonists and cholinergic antagonists.

56. Pharmaceutical composition according to Claim 55, **characterised in that** the dose of the additional active ingredient is decreased compared to the pharmaceutical composition containing solely one additional active ingredient.

57. Method for preparing the pharmaceutical composition for the treatment of noninfectious inflammatory diseases with neutrophil prevalence in people and animals including azithromycin as the active ingredient, its pharmaceutically acceptable derivative, its pharmaceutically acceptable hydrate, its pharmaceutically acceptable complex or chelate and its pharmaceutically acceptable salt, including mixing the active ingredient with additions and, if necessary, with other additional active ingredients useful for treating such diseases, dissolving or suspending the obtained mixture in a sterile water or water/alcohol solution, adjusting pH of the solution to a value from about 4 to 7 using agents for the pH adjustment, and filling into the bottles or ampoules.

58. Method according to Claim 57, **characterised in that** additional active ingredients are chosen from a group consisting of non-steroid anti-inflammatory agents, steroid anti-inflammatory agents, bronchodilators, antirheumatic agents, immunomodulators, immunosuppressive agents, corticosteroids, β2-agonists and cholinergic antagonists.

ABSTRACT

The present invention relates to the usage of the 9-deoxo-9-dihydro-9a-methyl-9a-aza-9a-homoerythromycin A (generic name: azithromycin) for treating noninfectious inflammatory diseases with neutrophil prevalence, pharmaceutical compositions containing azithromycin for the enteral or parenteral application, and methods for preparing those pharmaceutical compositions.

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(54) Title: NOVEL THERAPEUTIC INDICATION OF AZITHROMYCIN FOR TREATMENT OF NON-INFECTIVE INFLAMMATORY DISEASES

(57) Abstract: The invention relates to the use of 9-deoxo-9-dihydro-9a-methyl-9a-aza-9a-homoerythromycin. A (generic name: azithromycin) for the therapy of neutrophil-dominated non-infective inflammatory diseases, pharmaceutical compositions containing azithromycin for enteral or parenteral administration and methods for the production of these pharmaceutical composiitons.

Novel therapeutic indication of azithromycin for treatment of non-infective inflammatory diseases

Description

The invention relates to the use of 9-deoxo-9-dihydro-9a-methyl-9a-aza-9a-homoerythromycin A (generic name: azithromycin) for the therapy of neutrophil-dominated non-infective inflammatory diseases, pharmaceutical compositions containing azithromycin for enteral or parenteral administration and methods for the production of these pharmaceutical compositions.

Most inflammatory diseases are characterised by abnormal accumulation of inflammatory cells including monocytes/macrophages, granulocytes, plasma cells, lymphocytes and platelets. Along with tissue endothelial cells and fibroblasts, these inflammatory cells release a complex array of lipids, growth factors, cytokines and destructive enzymes that cause local tissue damage.

One form of inflammatory response is neutrophilic inflammation which is characterized by infiltration of the inflamed tissue by neutrophil polymorphonuclear leucocytes (PMN), which are a

major component of host defence. Tissue infection by extracellular bacteria represents the prototype of this inflammatory response. On the other hand, various non-infectious diseases are characterized by extravascular recruitment of neutrophils. This group of inflammatory diseases includes chronic obstructive pulmonary disease, adult respiratory distress syndrome, some types of immune-complex alveolitis, cystic fibrosis, bronchitis, bronchiectasis, emphysema, glomerulonephritis, active phases of rheumatoid arthritis, gouty arthritis, ulcerative colitis, certain dermatoses such as psoriasis and vasculitis. In these conditions neutrophils are thought to play a crucial role in the development of tissue injury which, when persistent, can lead to the irreversible destruction of the normal tissue architecture with consequent organ dysfunction. Thereby tissue damage is mainly caused by the activation of neutrophils followed by their release of proteinases and increased production of oxygen species.

Chronic obstructive pulmonary disease (COPD) is basically a condition described by the progressive development of airflow limitation that is not fully reversible (ATC, 1995). Most patients with COPD have three pathological conditions: bronchitis, emphysema and mucus plugging. This disease is characterised by a slowly progressive and irreversible decrease in forced expiratory volume in the first second of expiration (FEV_1), with relative preservation of forced vital capacity (FVC) *(Barnes, N. Engl. J. Med. (2000), 343(4): 269-280)*. In both asthma and COPD there is significant, but distinct, remodelling of airways. Most of the airflow obstruction is due to two major components, alveolar destruction (emphysema) and small airways obstruction (chronic obstructive

bronchitis). In COPD it is mainly characterised by profound mucus cell hyperplasia.

Cigarette smoking, air pollution and other environmental factors are major causes of the disease. The causal mechanism remains currently undefined but oxidant-antioxidant disturbances are strongly implicated in the development of the disease. COPD is a chronic inflammatory process that differs markedly from that seen in asthma, with different inflammatory cells, mediators, inflammatory effects and responses to treatment *(Keatings et al., Am. J. Respir. Crit. Care Med. (1996), 153: 530-534)*. Primarily, neutrophil infiltration of the patient's lungs is a characteristic of this disease.

Elevated levels of proinflammatory cytokines like TNF-α, and especially chemokines like IL-8 and GRO-α seem to play a very important role in pathogenesis of this disease. Platelet thromboxane synthesis is also enhanced in patients with COPD *(Keatings et al., Am.J. Respir. Crit. Care Med. (1996), 153: 530-534; Stockley and Hill, Thorax (2000), 55(7): 629-630)*. Most of the tissue damage is caused by activation of neutrophils followed by their release of (metallo)proteinases, and increased production of oxygen species *(Repine et al., Am. J. Respir. Crit. Care Med. (1997), 156: 341-357; Barnes, Chest (2000), 117(2 Suppl): 10S-14S)*.

Most therapeutic endeavour is directed towards the control of symptoms *(Barnes, Trends Pharm. Sci. (1998), 19(10): 415-423; Barnes, Am. J. Respir. Crit. Care Med. (1999) 160: S72-S79; Hansel et al., Expert Opin. Investig. Drugs (2000) 9(1): 3-23)*. Symptoms usually equate with airflow limitation and bronchodilators are the therapy of choice.

Prevention and treatment of complications, prevention of deterioration and improved quality and length of life are also primary goals stated in the three key international guidelines for the management of COPD *(Culpitt and Rogers, Exp. Opin. Pharmacother. (2000) 1(5): 1007-1020; Hay, Curr. Opin. Chem. Biol. (2000), 4: 412-419)*. Basically, most of the current therapeutic research has been focused on mediators involved in the recruitment and activation of neutrophils, or attenuation of consequences of their undesirable activation *(Stockley et al., Chest (2000), 117(2 Suppl): 58S-62S)*.

There are a number of reports on immunomodulatory action of macrolide antibiotics in vitro *(Labro, J. Antimicrob. Chemother. (1998), 41 (Suppl B): 37-46; Labro, Clin. Microb. Rev. (2000), 13(4): 615-650; Wales and Woodhead, Thorax (1999), 54 (Suppl 2): S58-S62)*. Macrolide antibiotics are macrocyclic compounds containing for example a 12-, 14-, 16- or 17-membered lactone ring and 1 to 3 sugar residues, which are linked to each other or to the aglucone by glycosidic bounds. Known members of macrolide antibiotics are for example carbomycin, erythromycin, leucomycin and spiramycin.

The most important findings with regard to macrolide interaction with phagocytic inflammatory cells *in vitro* concern the inhibitory effects on oxidant production by stimulated cells *(Labro et al., J. Antimicrob. Chemother. (1989), 24 (4): 561-572; Umeki, Chest (1993), 104: 1191-1193; Wenisch at al., Antimicrob. Agents Chemother. (1996), 40(9): 2039-2042)* and modulation of pro-inflammatory and anti-inflammatory cytokine release by these cells *(Labro et al., J. Antimicrob. Chemother. (1989), 24 (4): 561-572; Khan et al, Internat. J. Antimicrob. Agents. (1999), 11: 121-*

132; Morikawa et al., Antimicrob. Agents and Chemother. (1996), 40(6): 1366-1370; Sugiyama et al., Eur. Respir. J. (1999), 14: 1113-1116). In addition, several macrolides directly stimulate exocytosis (degranulation) by human neutrophils *in vitro (Abdelghaffae et al., Antimicrob. Agents Chemother. (1994), 38(7): 1548-1554; Vazifeh et al., Antimicrob. Agents Chemother. (1998), 42 (8): 1944-1951).* In the experimental inflammatory model of carrageenin pleurisy in the rat, some macrolide antibiotics like roxithromycin, clarithromycin and erythromycin, but not azithromycin, were found to show anti-inflammatory activity which probably depended on their ability to prevent the production of pro-inflammatory mediators and cytokines. In this model of acute inflammation, NO production, TNF-α levels or PGE_2 were significantly reduced by the antibiotic pre-treatment *(Ianario et al., J. Pharmacol. Exp. Ther. (2000), 292: 156-163).*

Erythromycin administration also caused anti-inflammatory effects in zymosan-induced peritonitis in rats *(Agen et al., Agents Actions (1993), 38(1-2): 85-90).* Roxithromycin was reported to be active in reducing the acute inflammatory reaction through a mechanism different from conventional anti-inflammatory agents such as indomethacin. In another study, roxithromycin was demonstrated to be effective in a standard animal model used for evaluating the effects of anti-inflammatory drugs on carrageenin-induced paw oedema, whereas clarithromycin and azithromycin showed modest activity *(Scaglione and Rossini, J. Antimicrob. Chemother. (1998), 41, Suppl B: 47-50).*

Some macrolide antibiotics, like erythromycin, clarithromycin and roxithromycin have already been used as anti-inflammatory drugs, especially for the treatment of diffuse panbronchiolitis. Reports on

the use of macrolides for diseases like rheumatoid arthritis and cystic fibrosis are available *(Arayssi et al., Programm and Abstracts of the 4th International conference on macrolides, azalides, streptogramins and ketolides, 21-23 January 1998, Barcelona, Spain, Abstract 6; Singh, J. Assoc. Phys. India (1989), 37: 547; Jaffe et al., Lancet (1998), 351: 420)*. With regard to relevant pharmacological effects of macrolides, it has been reported that erythromycin inhibits hypersecretion due to inhibition of mucus and water secretion from epithelial cells. It also inhibits neutrophil accumulation in the inflammatory region due to inhibition of their attachment to the capillary vessels, IL-8 secretion from the epithelial cells and secretion of IL-8 and LTB_4 from the neutrophil, itself. Its beneficial effects in diffuse panbronchiolitis also include a reduction of superoxide production, and reduction of the proteolytic enzyme levels in lungs.

Azithromycin has been shown to significantly improve lung function, but the underlying mechanism was unclear *(Jaffe et al., Lancet (1998), 351: 420)*, while roxithromycin was reported to suppress the growth of nasal polyp fibroblasts *(Nonaka et al., Am. J. Rhinol. (1999), 13: 267-272, Yamada et al., Am. J. Rhinol. (2000), 14: 143-148)*.

While strong evidence in published literature exists that macrolides with a 14-membered ring such as erythromycin, clarithromycin and roxithromycin inhibit in vitro IL-8 production and neutrophil chemotaxis, evidence even in vitro is limited that macrolides with a 15-membered ring such as azithromycin exert a similar anti-inflammatory action *(Criqui et al., Eur. Respir. J. (2000), 15: 856-862)*.

In US 4,886,792 inhibitory effects on neutrophil degranulation of 15-membered macrolactones were described, but these lacked the sugar substituents of azithromycin. Azithromycin has been reported to induce apoptosis in human neutrophils *in vitro,* but was without effect on oxidative metabolism or IL-8 production *(Koch et al., J. Antimicrob. Chemother. (2000), 46: 19-26).* Only one study has shown azithromycin to inhibit neutrophil chemotaxis and active oxygen generation *in vitro (Sugihara, Kansenshogaku Zasshi J. Jpn. Assoc. Infec. Dis. (1997), 71: 329-336).* Also, azithromycin has been shown not to change TNFα, IL-1β or IL-6 levels of alveolar macrophages or blood *(Aubert et al., Pul. Pharmacol. Ther. (1998), 11: 263-269).*

The possibility that azithromycin, by virtue of its 15-membered ring, lacks the requisite structure conferring anti-inflammatory activity to the 14-membered macrolides has been suggested and is made more likely by the observation that 16-membered macrolides such as josamycin do not reduce IL-8 production *(Takizawa et al., Am. J. Resp. Crit.Care Med. (1997), 156: 266-271; Criqui et al., Eur. Respir. J. (2000), 15: 856-862).*

In comparison with macrolide antibiotics having a 14-membered ring macrolide compounds with a 15-membered ring possess several advantages. For example erythromycin whose structure is characterised by a 14-membered aglucone ring is in acidic medium easily converted into anhydroerythromycin, which is an inactive C-6/C-12 metabolite of a spiroketal structure *(Kurath et al., Experienta (1971), 27: 362).* In contrast to its parent antibiotic erythromycin azithromycin exhibits an improved stability in acidic medium. Furthermore, azithromycin exhibits a significantly higher concentration in tissues. Due to its

improved in vitro activity against gram-negative microorganisms there was even tested the possibility of a one-day dose (*Ratshema et al., Antimicrob. Agents Chemother. (1987), 31: 1939*).

Thus, the technical problem underlying the present invention is to provide improved means, in particular improved processes and applications useful for the therapy of neutrophil-dominated non-infective inflammatory diseases, in which the active ingredient exhibits the advantageous anti-inflammatory activities of macrolide compounds having a 14-membered lactone ring as well as the improved stability and high tissue concentration of macrolide compounds having a 15-membered ring.

The present invention solves the above problem by the use of an active ingredient selected from the group consisting of azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt thereof, for the production of pharmaceutical compositions for the treatment of neutrophil-dominated, non-infective inflammatory diseases in human beings and animals.

In contrast to the limited effects of azithromycin on neutrophil function *in vitro* described in the art according to the present invention it has been surprisingly found that azithromycin administered to humans *in vivo* has a broad range of anti-inflammatory activities and is highly useful in the therapy of inflammatory diseases characterized by neutrophil infiltration and neutrophil associated tissue damage.

In a trial conducted on healthy volunteers the influence of azithromycin on selected inflammation-relevant parameters was followed up. Thereby it was found that the administration of azithromycin stimulates the degranulation of human neutrophils as shown by a strong change of the concentration of primary azurophilic granular enzymes, such as myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase.

The biological relevance of MPO activity in granulocytes is a strong oxygen-dependent antimicrobial activity connected to mobilisation of all granules in the inflammatory granulocytes in the inflammation process, especially after phagocytic stimulus by immune complexes. After azithromycin application MPO activities in blood smear neutrophils strongly decreased and returned to baseline only after 28 days. Thereby it was found that degranulation presented with lower MPO neutrophil density as determined with cytochemistry was associated with lower MPO ELISA concentrations in neutrophil lysates.

N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase are lyosomal enzymes, both of which are located in azurophilic (primary or peroxidase-positive) granules of neutrophils. Since during inflammation degranulation of neutrophils occurs, both enzymes are markers of degranulation and can be used for estimation of neutrophil reactivity. The studies on azithromycin showed that after azithromycin application the activity of NAGA in serum increased considerably. Even 28 days after the last azithromycin dose serum NAGA values were still 70% higher than initial values. The increase in NAGA in serum was accompanied by a decrease in enzyme activity in PMN. The activity of β-glucuronidase in serum did not show any changes

during the first day after the last azithromycin dose but afterwards increased. 28 days after the last dose of azithromycin the activity of β-glucuronidase was 40% higher than initially. Activities of β-glucuronidase in PMN decreased within the next hours after the last azithromycin dose but than increased. 28 days after the last azithromycin dose β-glucuronidase activity in PMN was much higher than initially.

Furthermore, according to the invention it was shown that azithromycin inhibits the generation of reactive oxygen species from stimulated neutrophils as demonstrated by the inhibition of chemiluminescence generated from stimulated neutrophils. That azithromycin is an inhibitor of neutrophil oxidative burst was further demonstrated by using a cytochrom c assay system. The studies also revealed that azithromycin has also a long-term effect on the concentration of cellular gluthatione peroxidase (GSHPx) and gluthatione reductase, two enzymes that control the biological effects of free radicals which have been implicated in the pathogenesis of a large number of diseases. Free radical production and disturbance in redox status can modulate the expression of a variety of inflammatory molecules, affecting certain cellular processes leading to inflammatory processes. Thus azithromycin provides a basis for the treatment of a variety of diseases such as COPD in which neutrophil radical production becomes excessive.

The studies also confirmed that azithromycin induces apoptosis, i.e. the programmed cell death, of certain cell types. Apoptosis is an important mechanism to complete an immune response. A three-day administration of azithromycin exerted a delayed pro-apoptotic effect on granulocytes, as indicated by the morphology of blood smear. The

number of apoptotic cells reached its maximum 28 days after the last azithromycin dose suggesting a decreased number of active, potentially damaging neutrophils.

In the study other anti-inflammatory effects of azithromycin were also detected. In contrast to previous studies *(Koch et al., J. Antimicrob. Chemother. (2000), 46: 19-26)* it was found according to the invention that azithromycin has a marked inhibitory effect on the release of IL-8 and also GRO-α. Interleukin-8 (IL-8) is a member of the neutrophil-specific CXC subfamiliy of chemokines. It is a potent neutrophil chemotactic and activating factor *(Oppenheim, Ann. Rev. Immunol. (1999), 9: 617)*. IL-8 is expressed in response to inflammatory stimuli. IL-8 delays spontaneous and TNF-α-mediated apoptosis of human neutrophils. In contrast to the effect on IL-8, azithromycin increases gradually the serum concentration of the cytokine IL-1, whereby the highest IL-1 concentration was found 24 h after the last azithromycin dose. However, the serum concentration of another cytokine, IL-6, was continuously decreased.

In contrast to earlier reports *(Semaan et al., J. Cardiovasc. Pharmacol. (2000), 36: 533-537)* in which azithromycin treatment did not significantly affect the plasma levels of soluble VCAM, studies conducted according to the present invention clearly showed a marked decrease of plasma levels of sVCAM already 24 h after azithromycin treatment.

The results obtained according to the invention demonstrate that a three-day treatment of healthy human subjects, with a standard antibacterial dosage regimen of azithromycin, exerts acute effects on neutrophil granular enzymes, oxidative

burst, oxidative protective mechanisms and neutrophil chemokines and circulating IL-1, IL-6, IL-8, as well as delayed effects on neutrophil apoptosis and soluble adhesion molecules.

According to the present invention, therefore, azithromycin can be used as a valuable prophylactic and/or therapeutic agent in neutrophil-dominated, non-infective inflammatory diseases.

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the present invention.

The term "neutrophil-dominated non-infective inflammatory disease" refers to inflammatory diseases, disorders or conditions which result from tissue damage, chemical irradiation or immune processes, but not from the invasion of microorganisms such as viruses, bacteria, fungi, protozoa or the like, und which are characterised by infiltration of the inflamed tissue by neutrophils which are the first inflammatory cells to enter the tissue and to amplify the inflammatory response. In some of non-infective inflammatory diseases neutrophils remain the dominant cell type within the inflamed area, even when the response is prolonged because of the continued presence of stimuli for neutrophil infiltration and activation. Examples therefore are chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS) and neutrophilic dermatoses. Other neutrophil-dominated non-infective inflammatory diseases include diseases which have an underlying stimulus to the chronicity of the pathology, which is not dependent on neutrophils. For example autoimmune diseases are mainly due to the development of immune responses

to normal structural components of the body and involve activation of T lymphocytes, with the possible production of autoantibodies by B lymphocytes. In rheumatoid arthritis (RA), for example, immune reactions are directed against structural components of the joints. However, in RA and other autoimmune diseases acute flare-ups occur, which are characterised by intense neutrophil infiltration and activation. These active phases of chronic autoimmune inflammation are neutrophil-dominated, for instance resulting in pronounced accumulation of neutrophils in the synovial fluids of patients with RA. In some autoimmune diseases, the generation of autoantibodies is pronounced, leading to deposition in the tissue of immune complexes of antigen and autoantibody and activation of the complement system. Neutrophils enter the tissue in an attempt to engulf the immune complexes and the neutrophil infiltration and activation is exacerbated by activated complement factors. An example of this type of disease is a renal disease, in particular glomerulonephritis resulting in pronounced kidney damage.

Therefore, the term "neutrophil-dominated non-infective inflammatory disease" includes, without being restricted to, chronic obstructive pulmonary disease (COPD), adult respiratory distress symptome (ARDS), bronchitis, bronchiectasis, emphysema, cystic fibrosis, inflammatory bowel disease, gouty arthritis, autoimmune diseases characterised by acute neutrophil-dominated phases, such as rheumatoid arthritis, autoimmune diseases, in which neutrophil infiltration is exacerbated by activated complement factors, such as glomerulonephritis, and skin diseases, in particular all kinds of neutrophilic dermatoses including psoriasisform dermatoses, such as psoriasis and Reiter's

syndrome, autoimmune bullous dermatoses, vessel-based neutrophilic dermatoses such as leukocytoclastic vasculitis, Sweet's syndrome, pustular vasculitis, erythema nodosum and familial Mediterranean fever, and pyoderma gangrenosum.

The term "neutrophil-dominated non-infective inflammatory disease" includes also all accompanying diseases, disorders or conditions which occur as a result of a neutrophil-dominated non-infective inflammatory disease and which can affect tissues or organs of the body other than that affected by the inflammatory disease itself. An example therefore are extraintestinal diseases such as uveitis and chronic hepatitis which can result from inflammatory bowel disease.

The term "active ingredient" or "active agent" refers to any substances which can affect or recognise biological cells or parts thereof, in particular cell organelles or cellular components. Such active ingredients or agents are of a chemical nature. In particular, such active ingredients or agents are diagnostics or therapeutics. In the context of the present invention the term "active ingredients" or "active agents" refers in particular to therapeutics, i.e. substances, which can be administered as a preventive measure or during the course of a disease, disorder or condition to organisms in need of such a treatment in order to prevent or to reduce or to abolish a disease, disorder or condition, in particular a neutrophil-dominated non-infective inflammatory disease.

In the context of the present invention, the term "treatment" refers to a prophylactic and/or therapeutic effect of a drug or medicament which in turn is defined as a pharmaceutical composition

comprising a pharmaceutically or diagnostically effective compound in combination with at least one additive, such as a carrier.

"Azithromycin" refers to the macrolide compound N-methyl-11-aza-10-deoxo-10-dihydroerythromycin A (9-deoxo-9-dihydro-9a-methyl-9a-aza-9a-homoerythromycin A) with a 15-membered azalactone ring which can be obtained by the Beckmann rearrangement of erythromycin A-oxime followed by Eschweiler-Clarke reductive N-methylation essentially as described in US 4,517,359, US 4,328,334 and BE 892,357, whereby the disclosure contents of these documents with regard to the methods for production of azithromycin are completely incorporated in the disclosure content of the present application.

The term "pharmaceutically acceptable derivative thereof" refers to non-toxic functional equivalents or derivatives of azithromycin, which can be obtained by substitution of atoms or molecular groups or bonds of the azithromycin molecule, whereby the basic structure of azithromycin is not changed, and which differ from the azithromycin structure in at least one position. The term "pharmaceutically acceptable derivative" includes for example O-methyl derivatives of azithromycin which can be obtained essentially as described in US 5,250,518, whereby the disclosure content of this document with regard to the methods for production of O-methyl derivatives is completely incorporated in the disclosure content of the present application.

The term "pharmaceutically acceptable derivative" includes also esters of azithromycin which retain, upon hydrolysis of the ester bond, the biological effectiveness and properties of azithromycin and are not biologically or otherwise undesirable. Techniques for the preparation of pharmaceutically acceptable esters are for instance disclosed in March Advanced Organic Chemistry, 3rd Ed., John Wiley & Sons, New York (1985) p. 1152. Pharmaceutically acceptable esters useful as prodrugs are disclosed in Bundgaard, H., ed., (1985) Design of Prodrugs, Elsevier Science Publishers, Amsterdam.

The term "pharmaceutically acceptable hydrate thereof" refers to non-toxic solid or fluid compounds of azithromycin retaining the biological activities of azithromaycin and generated by the process of hydration whereby one or more molecules of water associate with the azithromycin molecule due to dipole forces. The term includes for example mono- and dihydrates of azithromycin.

The term "pharmaceutically acceptable salts" refers to the non-toxic alkali metal, alkaline earth metal, and ammonium salts commonly used including the ammonium, barium, calcium, lithium, magnesium, potassium, protamine zinc salts and sodium, which are prepared by methods known in the art. The term also includes non-toxic; i.e. pharmaceutically acceptable acid addition salts, which are generally prepared by reacting azithromycin with a suitable organic or inorganic acid, such as acetate, benzoate, bisulfate, borate, citrate, fumarate, hydrobromide, hydrochloride, lactate, laurate,

maleate, napsylate, oleate, oxalate, phosphate, succinate, sulfate, tartrate, tosylate, valerate, etc.

The term "pharmaceutically acceptable acid addition salt" refers to salts which retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrobromic acid, hydrochloric acid, nitric acid, phosphoric acid, sulfuric acid, and organic acids such as acetic acid, benzoic acid, cinnamic acid, citric acid, ethanesulfonic acid, fumaric acid, glycolic acid, maleic acid, malic acid, malonic acid, mandelic acid, menthanesulfonic acid, oxalic propionic acid, p-toluenesulfonic acid, pyruvic acid, salicylic acid, succinic acid, tartaric acid, etc.

The salts of the invention can be obtained by dissolving azithromycin in an aqueous or aqueous/alcoholic solvent or in other suitable solvents with an appropriate base and then isolating the obtained salt of the invention by evaporating the solution, by freezing and lyophilization or by addition of another solvent, e.g. diethylether, to the aqueous and/or alcoholic solution of the azithromycin salt including the separation of unsoluble crude salt. For the preparation of alkali azithromycin salts, alkali metal carbonates or hydrogencarbonates are preferably used. The prepared salts are freely soluble in water.

The term "pharmaceutically acceptable complex or chelate thereof" refers to non-toxic complexes and chelates of azithromycin with bivalent and/or trivalent metals which can be obtained essentially as described in US 5,498,699, whereby the disclosure content of this document with regard to the methods for production of complexes and chelates of azithromycin is completely incorporated in the disclosure content of the present application. As complex- and chelate-forming metals, metals of the II and III group which can form physiologically tolerated compounds, in particular Mg^{2+}, Al^{3+}, Fe^{3+}, Rh^{3+}, La^{3+} and Bi^{3+} can be used. Preferably the ratio of azithromycin to metal is in the range of 1:1 to 1:4. In order to obtain complexes and chelates of azithromycin the antibiotic is reacted in form of a free base or salt, in particular as a hydrochloride, with a salt of a bivalent and/or trivalent metal in a ratio of 2:1 at ambient temperature in an aqueous solution or in a mixture of water/alcohol at a pH of 8,0 to 11,0 with a metal hydroxide and/or carbonate, subsalicylate or a gel thereof. Preferred examples include chelates of azithromycin with antacids chosen from the group of salts of Al, Mg and Bi, chelates of azithromycin with sucralfate and chelates of azithromycin with bismuth-subsalicylate which are in the form of a gel.

The term "pharmaceutically or therapeutically acceptable carrier" refers to a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredients and which is not toxic to the host or patient.

The active ingredient selected from the group consisting of azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically

acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt can also be administered to animals, including mammals such as rodents and primates, including humans, to prevent or to reduce or to abolish neutrophil-dominated non-infective inflammatory diseases. Thus, the present invention encompasses methods for therapeutic treatment of such disorders or diseases that comprise administering an active ingredient of the invention in amounts sufficient to reach the desired effect of azithromycin in vivo. For example, the active agent or ingredient of the present invention can be administered in a therapeutically or pharmaceutically effective amount to treat a variety of non-infective inflammatory diseases, including but not limited to COPD, ARDS and neutrophilic dermatoses.

"Therapeutically or pharmaceutically effective amount" as applied to azithromycin or the azithromycin containing compounds and compositions of the present invention refers to the amount of a compound or composition sufficient to induce a desired biological result. That result can be alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. In the present invention, the result will for instance in a particularly preferred embodiment involve preventing, abolishing and/or reducing the symptoms or causes of a neutrophil-dominated non-infective inflammatory condition by acute effects on neutrophil granular enzymes, oxidative burst, oxidative protective

mechanisms and neutrophil chemokines and circulating IL-1, IL-6, IL-8, as well as delayed effects on neutrophil apoptosis and soluble adhesion molecules. In a preferred embodiment the active ingredients of the present invention will be administered prophylactically prior to the outbreak of a neutrophil-dominated non-infective inflammatory disease.

Accordingly, the present invention also provides pharmaceutical compositions comprising, as an active ingredient azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt in association with a pharmaceutical carrier or diluent. The compositions of this invention can be administered systematically or topically, in particular by intravascular oral, pulmonary, parenteral, e.g. intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection or inhalation, e.g. via a fine powder formulation, transdermal, nasal, vaginal, rectal, or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration. The active agent or ingredient is adminstered preferably in a pharmaceutically effective amount.

Solid dosage forms for oral administration include capsules, lingualettes, tablets, pills, powders, liposomes, patches, time delayed coatings and granules. In such solid dosage forms, the active compound is admixed with at least one inert

pharmaceutically acceptable carrier such as lactose, sucrose, or starch. Such dosage forms can also comprise additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise bulking and/or buffering as well as flavouring agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, with the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as salts for varying the osmotic pressure, pH-adjusting compounds, skin penetration agents, wetting agents, emulsifying and suspending agents, and sweetening, flavouring, and perfuming agents.

Pharmaceutical compositions according to the present invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatine, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilised by, for example, filtration through a bacteria retaining filter, by incorporating sterilising agents into the compositions, by

irradiating the compositions, or by heating the compositions. They can also be manufactured using sterile water, or some other sterile injectable medium, immediately before use.

Formulations for injection will comprise a physiologically-acceptable medium, such as water, saline, PBS, aqueous ethanol, aqueous ethylene glycols and the like. Water soluble preservatives which may be employed include sodium bisulfite, sodium thiosulfate, ascorbate, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric borate, parabens, benzyl alcohol and phenylethanol. These agents may be present in individual amounts of from about 0.001 to about 5% by weight and preferably about 0.01 to about 2%. Suitable water soluble buffering agents that may be employed are alkali or alkaline earth carbonates, phosphates, bicarbonates, citrates, borates, acetates, succinates and the like, such as sodium phosphate, citrate, borate, acetate, bicarbonate and carbonate. Additives such as carbomethylcellulose may be used as a carrier in amounts of from about 0.01 to about 5% by weight. The formulation will vary depending upon the purpose of the formulation, the particular mode employed for treating a disease, the intended treatment, etc.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax. Compositions for nasal or sublingual administration are also

prepared with standard excipients well known in the art.

The compositions containing the active agent or ingredient of the present invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications, i.e. a therapeutically effective amount.

In prophylactic applications, compositions containing the active agent or ingredient of the present invention are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and weight.

The pharmaceutical compositions of the present invention may also be administered in the form of a depot, such as a slow release composition. Such a slow release composition may include particles of the active agent or ingredient in a matrix, made e.g. from collagen.

The quantities of the active agent or ingredient necessary for effective therapy will depend upon many different factors, including means of

administration, target site, physiological state of the patient, and other medicants administered.

The active agent or ingredient of the present invention selected from the group consisting of azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt thereof are effective in treating neutrophil-dominated non-infective inflammatory diseases when administered in a range of from about 10 mg to about 2000 mg per day, in particular from about 30 to about 1500 mg. The specific dose employed is regulated by the particular condition being treated, the route of administration, as well as by the judgement of the attending clinician depending upon factors such as the severity of the condition, and the age and general condition of the patient.

The active agents or ingredients of the present invention may be administered alone or together with other medicaments currently used for the treatment of neutrophil-dominated non-infective inflammatory diseases such as non-steroidal anti-inflammatory agents, such as methyl xanthine non-steroidal anti-inflammatory agents, steroidal anti-inflammatory agents, immunomodulating agents, immunosuppressive agents, bronchodilating agents, antirheumatic agents, corticosteroids, β2-agonists, cholinergic antagonists, and the like, whereby the dose of the latter can possibly be reduced by 50% or 25% due to the anti-inflammatory effects of the

active ingredients of the present invention.

The composition, preferably the water-soluble composition, of the invention may further contain a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity at the concentration used in one unit dosage form of the present invention (hereinafter, "water-soluble protein"). As such a water-soluble protein, serum albumin, globulin, collagen and/or gelatine are preferred. This protein can be added in an amount generally employed in injectable pharmaceutical compositions. Thus, for example, the weight ratio between the water-soluble protein and the active agent or ingredient of the present invention is about 0.0001:1 to 100:1, preferably about 0.001:1 to about 10:1 or more preferably about 0.01:1 to about 1:1.

Continuing, the invention also relates to the aforementioned active agents or ingredients themselves and compositions containing them, in particular, in dried and/or pure form or in an aqueous or aqueous/alcoholic solution. The pH of a solution prepared from the water-soluble composition or an active agent of the present invention should be such that said pH will not exert any adverse influence upon the activity of the pharmacologically active peptide, but is within an acceptable range for injections in general and further, such that said pH will neither cause a great change in viscosity of the solution nor allow formation of a precipitate or the like. Thus the

solution should preferably have a pH of about 4 to 7, preferably 5 to 6, in particular 5.3 to 5.5.

When the water-soluble composition of the invention is converted into an aqueous solution for administration, the concentration of the pharmacologically active agent or ingredient or salt thereof in said solution should preferably be about 0.0000001 to 10 % (w/v), more preferably about 0.000001 to 5% (w/v) or most preferably about 0.00001 to 1% (w/v).

The composition of the present invention should preferably have a unit dosage form containing the pharmacologically active agent or ingredient of the invention and, if necessary, together with further additives such as the above mentioned water-soluble protein. Thus, for example, the two or three components mentioned above are made to occur in an ampule or vial by dissolving or suspending them in sterile water or sterile physiological saline. In this case, the method of preparation may comprise admixing a solution of the pharmacologically active agent or ingredient and further, if necessary, a solution of the additive or adding the additive in a powder form to a solution of the pharmacologically active agent or ingredient or any other combination of adequate procedures. The dosage form may also be prepared by adding sterile water or sterile physiological saline to a lyophilizate or vacuum-dried powder in which the pharmacologically active agent, and if necessary the additive, coexist. This unit dosage form may contain one or more conventional additives such as

pH adjusting agents (e.g. glycine, hydrochloric acid, sodium hydroxide), local anesthetics (e.g. xylocaine hydrochloride, chlorobutanol), isotonizing agents (e.g. sodium chloride, mannitol, sorbitol), emulsifiers, adsorption inhibitors (e.g. Tween® 60 or 80), talcum, starch, lactose and tragacanth, magnesium stearate, glycerol, propylen glycol, preserving agents, benzyl alcohol, methylhydroxy benzoate and/or oleum arachid hydrogen. This unit dosage form may further contain pharmaceutically acceptable excipients such as polyethylene glycol 400 or dextran.

The composition of the present invention is made by admixing these ingredients according to a conventional method. The goal of admixing the ingredients of the present composition should be such that the activity of the pharmacologically active agent is maintained and bubble formation minimised during the process. The ingredients are put into a vessel (for example a bottle or drum) either at the same time or in any order. The atmosphere in the vessel can be, for example, sterile clean air or sterile clean nitrogen gas. The resultant solution can be transferred to small vials or ampules and can be further subjected to lyophilization.

The liquid form or the lyophilizate powder form of the composition of the present invention may be dissolved or dispersed in a solution of a biodegradable polymer such as poly(lactic-glycolic) acid copolymer, poly(hydroxybutyric acid), poly(hydroxybutyric-glycolic) acid copolymer, or

the mixture of these, and then may be formulated, for example, to films, microcapsules (microspheres), or nanocapsules (nanospheres), particularly in the form of soft or hard capsules.

In addition, the composition of the present invention encapsulated in liposomes comprising phospholipids, cholesterol or the derivatives of these can be further dispersed in physiological saline or a hyaluronic acid solution dissolved in physiological saline.

The soft capsule may be filled with the liquid form of the composition of the present invention. The hard capsule may be filled with the lyophilizate powder of the composition of the present invention, or the lyophilizate powder of the present composition may be compressed to tablets for rectal administration or oral administration respectively.

Of course, the composition of the present invention can be supplied in a pre-filled syringe for self-administration.

Although only preferred embodiments of the invention are specifically described above, it will be appreciated that modifications and variations of the invention are possible without departing from the spirit and intended scope of the invention. Further preferred embodiments of the present invention are listed in the claims.

Example

A trial on healthy volunteers was conducted and the

influence of azithromycin given in a dosage of 3 x 500 mg on selected inflammation-relevant parameters was followed up.

Drug administration, blood sampling and plasma

Each subject received two standard 250 mg capsules of azithromycin (Sumamed®, PLIVA Zagreb) on three consecutive days. Immediately before the treatment and 2h and 30min, 24h and 28 days after the third and last dose of azithromycin blood was collected from the cubital vein into EDTA-containing tubes. Aliquots were taken for cell counting, smear preparation, polymorphonuclear cell and serum isolation.

Analysis of primary azurophilic granular enzymes

Leucocyte granules are membrane-bound organells containing an array of antimicrobial proteins. Apart from containing degradative enzymes that may be extracellulary secreted from the neutrophil or else discharged into phagocytic vesicles, the membranes of many types of these granules and vesicles contain important molecules such as certain receptors (e.g. fMLP receptor) and cytochrome b of NADPH oxidase.

a) Analysis of myeloperoxidase

The enzyme myeloperoxidase (MPO) is a 135,000 dalton protein containing two heavy and two light chains of 55,000 and 15,000 daltons. MPO is

situated in primary or azurophil granules of granulocytic cells. The function of MPO is to provide reactive oxygen metabolites that are essential for microbicidal activity of neutrophils. The generation of oxygen metabolites is dependent on components of MPO-negative granules (which harbour the flavocytochrome b_{558}, an essential component of the NADPH oxidase) and on components of azurophil MPO positive granules. MPO transforms the relatively innocuous product of the NADPH oxidase, H_2O_2, to hypochlorous acid. The biological relevance of MPO activity in granulocytes is a strong oxygen-dependent antimicrobial activity connected to mobilisation of all granules in the inflammatory granulocytes in the inflammation process, especially after phagocytic stimulus by immune complexes.

The activity of MPO was assessed from the intensity of staining of neutrophils in blood smears and in cell lysates by ELISA. After fixation in ethanol-formaldehyde, smears were incubated in a substrate solution containing hydrogen peroxide and benzidine (SIGMA). After incubation, smears were counterstained with Giemsa solution. MPO value positivity of 100 granulocytes was evaluated and scored from 0 to 4+ on the basis of the intensity of the precipitated dye in cytoplasm. Therefore, the value of the score could be from 0 to 400. Normal values of score range (290-390) were taken from this study before azithromycin administration. MPO activity was also evaluated on the digital image of smear taken with a digital camera under the high magnification (x 1000) of light

microscope. MPO activities in blood smear neutrophils decreased from 2h and 30 min to 24h after the last azithromycin dose and returned to baseline after 28 days (Table 1). The concentration of MPO enzyme protein determined by ELISA in lysates of neutrophils is shown in Table 1. The change in neutrophil enzyme protein followed the same pattern as that in intracellular enzyme activity, decreasing from 2h and 30min to 24h after the last dose of azithromycin and returning to baseline after 28 days. Both methodological approaches of MPO determination confirmed each other. Degranulation presented with lower MPO neutrophil density determined with cytochemistry was associated with lower MPO ELISA concentrations in neutrophil lysates.

b) Analysis of N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase

Glycosidases are enzymes that catalyse hydrolysis of glycosidic bonds of oligosacharides and other glycosides. They are specific to the glycosidic part of substrate molecule. N-acetyl-β-D-glucosaminidase (NAGA) and β-glucuronidase are such enzymes. They are lysosomal enzymes, both located in azurophilic (primary; peroxidase-positive) granules of neutrophils. Since degranulation of neutrophils is present during inflammation, many authors choose these enzymes as markers of degranulation and for estimation of neutrophil reactivity. The catalytic concentration of both enzymes in serum and in neutrophil lysates was determined using the fluorimetric method described

by O'Brien et al. *(New Engl. J. Med. (1970) 283: 15-20)* for NAGA and Glaser & Sly *(J. Lab. Clin. Med. (1973) 82: 969)* for β-glucuronidase.

The results showed (Table 1) that activity of NAGA in serum increased about 30% 2h and 30min after the last dose. 24 hours after the last dose it was approximately 50% higher than the initial values. 28 days later, serum NAGA values were still 70% higher than initial values. The increase in NAGA in serum was accompanied by a decrease in enzyme activity in PMN. 2h and 30 min after the last dose a decrease of about 70% in NAGA in granulocytes was determined. 24 hours later, NAGA activity in PMN increased by about 30% but it was still about 40% lower compared to initial values. After 28 days the activity of NAGA increased 40% over the initial values (Table 1).

The activity of β-glucuronidase in serum did not show any changes during the first 24 hours after the last dose. 28 days later serum values were about 40% higher than initially. Activities of β-glucuronidase in PMN decreased by about 20% after 2h and 30 min and by about 50% 24 hours later compared to initial values. However 28 days later, β-glucuronidase activity in PMN was much higher (about 300%) compared to the initial values (Table 1).

When analysing activities of glycosidases it is obvious that azithromycin in healthy volunteers induced release of 40 - 50% enzymes from azurophilic granules within 24 hours after the last

dose. The decrease of NAGA activity in PMNs was accompanied by an increase in serum. Serum activities of the two enzymes showed a slight increase over baseline (before azithromycin) 2h and 30 min and 24h after the last dose of the drug, increasing a further 28 days later (Table 1).

In contrast, activities of the two enzymes in neutrophil lysates decreased in the hours after the last dose of azithromycin, the fall in NAGA activity being maximal after 2h and 30 min and returning to baseline after 28 days. The cellular activity of β-glucuronidase was still falling 24h after the last dose of azithromycin and increased to well above baseline levels after 28 days (Table 1).

In summary, enzymes released from neutrophil primary azurophilic granules tended to be present in serum at slightly higher activities 2 h and 30 min to 24h after azithromycin administration, while over the same time period, their activities were lower in peripheral blood neutrophils, suggesting that they were being released by degranulation. NAGA was released early after azithromycin, while MPO and β-glucuronidase exhibited a delayed release. Recovery of these enzyme activities also varied.

Studies on neutrophil oxidative burst

All aerobic organisms use oxygen for the production of energy. However, there are many indications that the advantages of using oxygen are associated with

a risk that the oxidative process may also cause injury. During phagocytosis when neutrophils are stimulated, they undergo an oxidative burst, with generation and release of reactive oxygen metabolites. These reactive oxygen species serve as the major mechanism by which phagocytes mediate their antimicrobial effect. The reactions are characterised by rapid oxygen uptake followed by reduction of oxygen to superoxide (O_2^-). This is catalysed by NADPH oxidase using NADPH or NADH as electron donor. When these defence mechanisms are directed inappropriately, tissue damage occurs.

a) Determination of chemiluminescence generation

The generation of reactive oxygen species by activated cells is frequently determined by the measurement of chemiluminescence (CL). The radical species formed react with a photon-producing chemical (e.g. Luminol) and the resulting light emission is measured with a photocell. Chemiluminescence is detectable as a result of the stimulation (e.g. fMLP) of leucocytes and is a measure of their oxidative cytotoxic activity *(Allen et al., Biochem. Biophys. Res. Commun. (1972), 47: 679)*.

The results of the study presented in Table 1 show, that azithromycin inhibits chemiluminescence generated from stimulated neutrophils isolated from the blood of humans treated with azithromycin.

b) Cytochrome c assay system

Neutrophils were incubated with cytochrome c and stimulated with fMLP (*Cohen and Chovaniec, 1978, J. Clin. Invest. 61: 1081-1087)*. Absorbances at 550 nm and 540 nm were recorded and the results were expressed as delta A.

The oxidative burst of neutrophils in response to the bacterial peptide fMLP was inhibited by the 3 day dosing with azithromycin (Table 1). Using both cytochrome c and luminol as assay systems, inhibition was already detectable 2h and 30 min after the last dose of azithromycin, was greater after 24h and had not returned to normal 28 days later.

Consequently, azithromycin is to be considered as an inhibitor of the oxidative burst. Thus, azithromycin provides a basis for a variety of diseases in which neutrophil radical production (oxidative burst) becomes excessive such as COPD.

Analysis of glutathione peroxidase and glutathione reductase

Oxygen free radicals and lipid peroxides have been implicated in the pathogenesis of a large number of diseases. The biological effects of free radicals are controlled *in vivo* by a wide range of antioxidants such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene, reduced glutathione (GSH) and antioxidant enzymes (superoxide dismutase, SOD, glutathione peroxidase GSHPx, catalase, CAT) *(Benabdeslam et al., Clin. Chem. Lab. Med. (1999), 37: 511-516; Mates et al.,*

Blood Cells Mol. (1999), 25: 103-109). Recently, antioxidant functions have been definitively linked to anti-inflammatory and/or immunosuppressive properties *(Mates et al., Blood Cells Mol. (1999), 25: 103-109)*. Free radical production and disturbance in redox status can modulate the expression of a variety of inflammatory molecules *(Sundaresan et al., Science (1995), 270: 296-299; Kaouass et al., Endocrine (1997), 6: 187-194)*, affecting certain cellular processes leading to inflammatory processes, both exacerbating inflammation and effecting tissue damage *(Tsai et al., FEBS Lett. (1997), 436: 411-414)*.

Cellular glutathione peroxidase (GSHPx) is a tetrameric protein in which each of the four identical subunits contains one atom of selenium (Se) in the form of selenocysteine at the active site *(Misso et al., J. Leukoc. Biol. (1998), 63: 124-130)*. GSHPx plays a role in H_2O_2 detoxification and converts lipid hydroperoxides to nontoxic alcohols *(Akkus et al., Clin. Chim. Acta (1996), 244: 221-227); Urban et al., Biomed & Pharmacother. (1997), 51: 388-390)*. In this study, in healthy volunteers treated with azithromycin alterations in the PMN intracellular GSHPx activity were determined using the commercially available kit RANSEL (Randox Laboratories). GSHPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidised glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

Glutathione reductase is an ubiquitous enzyme that catalyses the reduction of oxidised glutathione (GSSG) to glutathione (GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. GSH serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the GSHPx and glutathione S-transferases in the detoxification of organic peroxides and metabolism of xenobiotics. Glutathione reductase was determined using the BIOXYTECH® GR-340™ colorimetric assay for glutathione reductase (OXIS International, Inc.). Briefly, oxidation of NADPH to $NADP^+$ is catalysed by a limiting concentration of glutathione reductase.

GSHPx activity in neutrophil lysates (expressed per number of cells) was unchanged 2h and 30 min after the last dose of azithromycin, but decreased significantly 24h after this last dose (Table 1). The activity had returned to baseline 28 days later. Glutathione reductase activity in cell lysates (expressed per number of cells) showed a similar tendency, decreasing significantly 2 and 30 min and 24 h after the last dose of azithromycin, returning to normal values and then reaching higher levels than normal 28 days after the treatment (Table 1).

Analysis of apoptosis

Three-day administration of azithromycin exerted a delayed pro-apoptotic effect on granulocytes, as

indicated by morphology of blood smears. The results are presented in Table 1. The number of apoptotic cells counted increased continuously after the three day dosing with azithromycin, achieving statistical significance 28 days after the last dose. An increased number of apoptotic cells suggest a decreased number of active, potentially damaging neutrophils.

Analysis of cytokines and chemokines

Other acute, but potentially anti-inflammatory effects of azithromycin were also detected in this study.

Interleukin-8, a member of the neutrophil-specific CXC subfamily of chemokines is a potent neutrophil chemotactic and activating factor *(Oppenheim, J.J. Ann. Rev. Immunol. (1999), 9: 617)*. It binds to at least two G protein-coupled receptors (IL-8R1 and IL-8R2). These receptors are functionally different. Responses, such as cytososlic free Ca^{2+} changes and release of the granule enzymes, are mediated through both receptors, whereas the respiratory burst and the activation of phospholipase D depend exclusively on stimulation through IL-8R1 *(Johnes et al., Proc. Natl. Acad. Sci. USA (1996), 93: 6682-6686)*. IL-8 is a key mediator in the recruitment of circulating neutrophils. This chemokine is expressed in response to inflammatory stimuli, and is secreted by a variety of cell types, including lymphocytes, epithelial cells, keratinocytes, fibroblasts, endothelial cells, smooth muscle cells and

neutrophiles. In the latter instance, IL-8 is one of the most abundantly secreted (and most extensively) studied cytokines produced by neutrophils. Interestingly enough, neutrophils represent the primary cellular target for IL-8, to which they respond by chemotaxis, release of granule content, respiratory burst, up-regulation of cell surface receptors, increased adherence to non-stimulated endothelial cells, and transmigration across the endothelium. Agents capable of stimulating the production of IL-8 by human neutrophils are: TNF-α, IL-1β, GM-CSF, leukotriene B_4, PAF, fMLP, lactoferrin, LPs and many others *(Cassatella, M.A., Adv. Immunol. (1999), 73: 369-509)*. IL-8 delays spontaneous and TNF-α-mediated apoptosis of human neutrophils. *(Kettritz et al., Kidney Int. (1998), 53: 84-91)*. IL-8 is the pre-dominant C-X-C chemokine and the dominant neutrophil chemoattractant accumulating in supernatant of LPS-stimulated human alveolar macrophages *(Goodmann et al., Am. J. Physiol. (1998), 275: L87-L95)*.

Erythromycin was reported to have an inhibitory effect on IL-8 expression in human epithelial cells and this mode of action is probably of relevance for its clinical effectiveness *(Takizawa et al., Am. J. Respir. Crit. Care Med. (1997), 156: 266-271)*.

Roxithromycin is also capable of reducing IL-8 production in nasal polyp fibroblasts *(Nonaka et al., Acta Otolaryngol. (1998) Suppl. 539: 71-75)*. In synoviocytes from rheumatoid arthritis, the

production of IL-1α, IL-6, IL-8, GM-CSF could be inhibited by chlarithromycin *(Matsuoka et al., Clin. Exp. Immunol. (1996), 104(3): 501-8). Ex vivo* assessment of IL-8 production in whole blood also confirmed the potential of erythromycin for inhibiting IL-8 production *(Schultz et al., J. Antimicrob. Chemother. (2000), 46: 235-240.* A similar finding has recently been reported for human bronchial epithelial cells *(Desaki M. et al., Biochim. Biophys Res. Commun. (2000) 267: 124-128).* A recent study, however, reported a lack of azithromycin modulatory effect on IL-8 production of PMN *in vitro (Koch et al., J. Antimicrob. Chemother. (2000), 46: 19-26).*

Cytokine and chemokine concentrations were determined using ELISA kits. Several different response patterns were seen in serum cytokine and chemokine concentrations following three-day administration of azithromycin. Rapid and pronounced decreases in the plasma concentrations of the neutrophil-stimulating chemokine, IL-8, and GRO-α were observed 2h and 30 min and 24h after the last dose of azithromycin (Table 1. The concentration of IL-8 returned essentially to baseline after 28 days, while that of GRO-□ was decreased at this time.

These data clearly demonstrate the acute inhibitory effect of azithromycin on the release of IL-8 *ex vivo,* extending this property also to inhibition of the release of the chemokine GRO-□. It should be stated, however, that the serum chemokine concentration was measured. Therefore one cannot

draw any conclusion as to the cellular source(s) of the chemokines.

The low baseline serum concentration of IL-1 gradually increased after the last dose of azithromycin, achieving statistical significance after 24h (Table 1). The concentration had returned to baseline 28 days after azithromycin. In contrast, the serum concentration of 1L-6 exhibited a continuous decrease, achieving statistical significance 28 days after the last dose of azithromycin (Table 1).

Analysis of adhesion molecules

In contrast to earlier reported data *(Semaan et al., J. Cardiovasc. Pharmacol. (2000), 36: 533-537)* in which azithromycin treatment did not significantly affect the plasma levels of soluble VCAM, in this study a decrease in serum sVCAM was observed 24h after the last dose of azithromycin, remaining significantly reduced after 28 days, indicating that azithromycin has the potential to inhibit both the generation of neutrophil chemotactic peptides and the expression and release of adhesion molecules for activated leucocytes (Table 1). For quantitative determination of serum concentration of human sVCAM, an ELISA kit was used (R&D systems, UK).

Proteins in PMN samples were determined according to the method of Bradford *(Anal. Biochem. (1976) 72: 248-254)* using bovine serum albumin as a standard.

Claims

1. Use of an active ingredient selected from the group consisting of azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt thereof, for the production of pharmaceutical compositions for the treatment of neutrophil-dominated, non-infective inflammatory diseases in human beings and animals.

2. Use according to claim 1, whereby the neutrophil-dominated, non-infective inflammatory disease is a pulmonary disease including chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS), bronchitis, bronchiectasis, cystic fibrosis and emphysema.

3. Use according to claim 1, whereby the neutrophil-dominated, non-infective inflammatory disease is a skin disease, in particular a neutrophil dermatosis including psoriasisform dermatoses such as psoriasis and Reiter's syndrome, autoimmune bullous dermatoses, vessel-based neutrophilic dermatoses such as leukocytoclastic vasculitis, Sweet's syndrome, pustular vasculitis, erythema nodosum and familial Mediterranean fever, and pyoderma gangrenosum.

4. Use according to claim 1, whereby the neutrophil-dominated, non-infective inflammatory disease is an autoimmune disease, in which neutrophil infiltration is exacerbated by activated complement factors, in particular a renal disease including glomerulonephritis.

5. Use according to claim 1, whereby the

neutrophil-dominated, non-infective inflammatory disease is an intestinal disease including inflammatory bowel disease.

6. Use according to claim 1, whereby the neutrophil-dominated, non-infective inflammatory disease is an autoimmune disease characterised by acute neutrophil-dominated phases, such as rheumatoid arthritis.

7. Use according to any one of claims 1 to 6, whereby the active ingredient is a O-methyl-derivative of azithromycin.

8. Use according to any one of claims 1 to 6, whereby the active ingredient is an ester of azithromycin.

9. Use according to any one of claims 1 to 6, whereby the active ingredient is a monohydrate of azithromycin.

10. Use according to any one of claims 1 to 6, whereby the active ingredient is a dihydrate of azithromycin.

11. Use according to any one of claims 1 to 6, whereby the active ingredient is a complex or chelate of azithromycin with metal ions.

12. Use according to claim 11, whereby the ratio between azithromycin to metal is 1:1 to 1:4.

13. Use according to claim 11 or 12, whereby the metal ions are bivalent metal ions.

14. Use according to claim 11 or 12, whereby the metal ions are trivalent metal ions.

15. Use according to any one of claims 1 to 6,

whereby the active ingredient is an alkali metal, alkaline earth metal, or an ammonium salt of azithromycin.

16. Use according to any one of claims 1 to 6, whereby the active ingredient is an acid addition salt of azithromycin.

17. Use according to claim 16, whereby the acid addition salt is formed with an inorganic acid.

18. Use according to claim 16 or 17, whereby the inorganic acid is hydrobromic acid, nitric acid, phosphoric acid or sulphuric acid.

19. Use according to claim 16, whereby the acid addition salt is formed with an organic acid.

20. Use according to claim 19, whereby the organic acid is acetic acid, benzoic acid, cinnamic acid, citric acid, ethanesulfonic acid, fumaric acid, glycolic acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, oxalic acid, p-toluenesulfonic acid, pyruvic acid, salicylic acid, succinic acid or tartaric acid.

21. Use according to any one of claims 1 to 20, whereby the pharmaceutical compositions contain the active ingredient in an amount sufficient to abolish or to reduce the disease or to stop its progression.

22. Use according to claim 21, whereby the pharmaceutical compositions are administered one to three times a day in a dose of 10 mg to 2000 mg active ingredient.

23. Use according to claim 22, whereby the pharmaceutical compositions are administered one to three times a day in a dose of 30 mg to 1500 mg

active ingredient.

24. Use according to any one of claims 1 to 23, whereby the pharmaceutical compositions are orally administered in solid or liquid dosage forms.

25. Use according to claim 24, whereby the solid pharmaceutical compositions for oral administration are capsules, lingualettes, tablets, pills, powders, liposomes, patches, time delayed coatings and granules.

26. Use according to claim 24 or 25, whereby the solid pharmaceutical compositions for oral administration contain at least one inert pharmaceutically acceptable carrier.

27. Use according to claim 26, whereby the inert pharmaceutical carrier is lactose, sucrose, or starch.

28. Use according to any one of claims 24 to 27, whereby the solid pharmaceutical compositions for oral administration comprise additional substances selected from the group consisting of lubricating agents such as magnesium stearate, bulking and/or buffering agents and flavouring agents.

29. Use according to any one of claims 24 to 28, whereby the solid pharmaceutical compositions for oral administration are prepared with enteric coatings.

30. Use according to claim 24, whereby the liquid pharmaceutical compositions for oral administration are pharmaceutically acceptable emulsions,

solutions, suspensions or syrups.

31. Use according to claim 30, whereby the liquid pharmaceutical composition for oral administration contains at least one inert pharmaceutical carrier.

32. Use according to claim 31, whereby the inert pharmaceutical carrier is water or physiological saline.

33. Use according to any one of claims 30 to 32, whereby the liquid pharmaceutical composition for oral administration comprises additional substances, selected from the group consisting of adjuvants, salts for varying the osmotic pressure, pH-adjusting compounds, skin penetration agents, wetting agents, emulsifying and suspending agents.

34. Use according to any one of claims 1 to 23, whereby the pharmaceutical compositions are parenterally administered.

35. Use according to claim 34, whereby the pharmaceutical compositions for parenteral administration are infusions or injections.

36. Use according to claim 34 or 35, whereby the pharmaceutical compositions for parenteral administration are sterile aqueous or non-aqueous solutions, suspensions or emulsions.

37. Use according to any one of claims 34 to 36, whereby the pharmaceutical compositions for parenteral administration comprise non-aqueous solvents or vehicles.

38. Use according to claim 37, whereby the non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive

oil and corn oil, gelatine, and injectable organic esters such as ethyl oleate.

39. Use according to any one of claims 34 to 38, whereby the pharmaceutical compositions for parenteral administration comprise adjuvants such as preserving, wetting, emulsifying, and dispersing agents.

40. Use according to any of claims 1 to 23, whereby the pharmaceutical compositions are rectally or vaginally administered.

41. Use according to claim 40, whereby the pharmaceutical compositions for rectal or vaginal administration are suppositories, clysters or foams.

42. Use according to claim 40 or 41, whereby the pharmaceutical compositions for rectal or vaginal administation contain excipients such as cocoa butter or a suppository wax.

43. Use according to any one of claims 1 to 42, whereby the pharmaceutical compositions for the treatment of neutrophil-dominated, non-infective inflammatory diseases contain one or more additional active ingredients useful for the treatment of such diseases selected from the group consisting of non-steroidal anti-inflammatory agents, steroidal anti-inflammatory agents, bronchodilating agents, antirheumatic agents, immunomodulating agents, immunosuppressive agents, corticosteroids, β2-agonists and cholinergic antagonists.

44. Use according to claim 43, whereby the dose of the additional active ingredients is reduced in comparison to pharmaceutical compositions,

containing exclusively one of the additional active ingredients.

45. Pharmaceutical composition for the treatment of neutrophil-dominated, non-infective inflammatory diseases in human beings and animals comprising as active ingredient azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt thereof.

46. Pharmaceutical composition according to claim 45, whereby the active ingredient is an O-methyl-derivative or an ester of azithromycin.

47. Pharmaceutical composition according to claim 45, whereby the active ingredient is a monohydrate or a dihydrate of azithromycin.

48. Pharmaceutical composition according to claim 45, whereby the active ingredient is a complex or chelate of azithromycin with bivalent or trivalent metal ions.

49. Pharmaceutical composition according to claim 48, whereby the ratio between azithromycin and metal ions is 1:1 to 1:4.

50. Pharmaceutical composition according to claim 45, whereby the active ingredient is an alkali metal, alkaline earth metal, or an ammonium salt of azithromycin.

51. Pharmaceutical composition according to claim 45, whereby the active ingredient is an acid addition salt of azithromycin.

52. Pharmaceutical composition according to claim

51, whereby the acid addition salt is formed with an inorganic acid, such as hydrobromic acid, nitric acid, phosphoric acid or sulphuric acid.

53. Pharmaceutical composition according to claim 51, whereby the acid addition salt is formed with an organic acid, such as acetic acid, benzoic acid, cinnamic acid, citric acid, ethanesulfonic acid, fumaric acid, glycolic acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, oxalic acid, p-toluenesulfonic acid, pyruvic acid, salicylic acid, succinic acid or tartaric acid.

54. Pharmaceutical composition according to any one of claims 45 to 53, whereby the active ingredient is contained in an amount sufficient to abolish or to reduce the disease or to stop its progression.

55. Pharmaceutical composition according to any one of claims 45 to 54, comprising one or more additional active ingredients useful for the treatment of such diseases selected from the group consisting of non-steroidal anti-inflammatory agents, steroidal anti-inflammatory agents, bronchodilating agents, antirheumatic agents, immunomodulating agents, immunosuppressive agents, corticosteroids, β2-agonists and cholinergic antagonists.

56. Pharmaceutical composition according to claim 55, whereby the dose of the additional active ingredients is reduced in comparison to pharmaceutical compositions, containing exclusively one of the additional active ingredients.

57. Method for the production of a pharmaceutical composition for the treatment of neutrophil-dominated, non-infective inflammatory diseases in

human beings and animals comprising as an active ingredient azithromycin, a pharmaceutically acceptable derivate thereof, a pharmaceutically acceptable hydrate thereof, a pharmaceutically acceptable complex or chelate thereof and a pharmaceutically acceptable salt thereof comprising admixing the active ingredient with additives and optionally with additional active ingredients useful for the treatment of such diseases, dissolving or suspending the resulting admixture in sterile aqueous or aqueous/alcoholic solution, adjusting the pH of the solution to a value of about 4 to 7 by the use of pH adjusting agents and filling into vials or ampules.

58. Method according to claim 57, whereby the additional active ingredients are selected from the group consisting of non-steroidal anti-inflammatory agents, steroidal anti-inflammatory agents, bronchodilating agents, antirheumatic agents, immunomodulating agents, immunosuppressive agents, corticosteroids, β2-agonists and cholinergic antagonists.

Table 1.	UNITS	baseline	2 h and 30 min	24 hours	28 days
DEGRANULATION					
myeloperoxidase (score)		337±29	326±26	315±22*	347±18
myeloperoxidase (density)		105±13	130±16*	131±17*	115±19
myeloperoxidase (PMN)	µg/mg protein	54.22±12.61	70.85±19.91	26.74±2.51*	70.01±17.62
NAGA (PMN)	nmol x 10^{-6} cells x min^{-1}	4.15±01.6	1.13±0.72*	2.62±1.6*	5.95±3.7
β-glucuronidase(PMN)	nmol x 10^{-6} cells x min^{-1}	4.12±2.7	3.21±2.3	1.58±0.4*	15.37±11.4*
NAGA (serum)	µmol x L^{-1}x min^{-1}	9.16±1.6	11.52±2.2	13.7±1.5*	14.87±1.9*
β-glucuronidase (serum)	µmol x L^{-1}x min^{-1}	2.88±0.7	3.01±0.6	2.95±0.5	3.93±1.2*
CYTOKINES (serum)					
IL-1	pg/mL	0.291±0.11	0.533±0.15	1.07±0.19*	0.29±0.20
IL-6	pg/mL	3.4±1.05	2.7±1.49	2.5±1.48	1.15±0.61*
CHEMOKINES (serum)					
IL-8	pg/mL	29.47±15.44	10.61±3.81*	14.60±10.75*	23.03±19.72
GRO-α	pg/mL	124.1±33.02	109.6±30.35*	107.9±27.83*	90.4±22.32*
APOPTOSIS (WBC)	apoptopic cells/1000WBC	0.333±0.655	0.833±1.029	1.417±1.240	2.583±2.02*
ADHESION MOLECULES					
sV-CAM	ng/mL	13.59±2.90	12.21±4.12	10.29±2.12*	10.74±2.05*
OXIDATIVE BURST (PMN)					
fMLP-luminol	A.U.	29335±1957	14774±1175*	5053±3804*	9879±13880*
fMLP-cytochrom c	ΔA	0.020±0,014	0,007±0.015*	-0,018±0.010*	-0,0011±0,0010*
GLUTATHION PEROXIDASE (PMN)	mU/10^6 PMN	5.3±2.0	5.3±2.9	1.6±1.3 *	8.0±5.2
GLUTATHION REDUCTASE (PMN)	mU/10^6 PMN	9.63±1.16	7.39±1.23*	7.91±0.87*	11.27±2.24*

*p< 0.01 vs baseline (Wilcoxon).

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